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Remediation of an Oil Contaminated Wetland and the Effects of Crude Oil and Brine on Two-Year Old Loblolly Pine (Pinus Taeda) Seedlings.

Dean Anthony Goodin
Louisiana State University and Agricultural & Mechanical College

REMEDIATION OF AN OIL CONTAMINATED WETLAND AND THE EFFECTS OF CRUDE OIL AND BRINE ON TWO-YEAR OLD LOBLOLLY PINE (*PINUS TAEDA*) SEEDLINGS

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Dean Anthony Goodin
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Bell & Howell Information and Learning Company
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ABSTRACT

The goal of this project was to introduce a successful remediation and restoration plan for a wetland and pine forest contaminated by an oil well blowout. A field study focused on the effectiveness of ammoniated bagasse (ABG) to enhance the bioremediation of the contaminated wetland. A soil and vegetation greenhouse study investigated the effects of oil and brine on loblolly pine tree seedlings and the effectiveness of ABG to remove and remediate oil contaminated forest soils.

The most effective, ecologically sound, and economical plan to remove the oil from the wetland was to burn the area. Once the area was burned a combination of ABG, lime, and topsoil was applied *in situ* to 20 research plots in order to monitor the effectiveness of ABG to remediate the soils. The soil total petroleum hydrocarbon results of the study question the ability of ABG to remediate the site, as there was no statistically significant difference between treatments. The spatial variability of oil within the wetland made it difficult to prove the effectiveness ABG to promote bioremediation of the oil contaminated soils. Suggestions for further studies are provided.

Greenhouse studies investigated the effects of foliar and soil applications of unweathered crude oil and brine on two-year old loblolly pine seedlings. Results show that soil applied oil and brine had a greater negative effect (i.e. stress, death) than foliar applied oil and brine. A single short-term foliar application of brine had little or no effect on the seedlings. Foliar applied oil caused seedlings to show signs of stress when needle surface area coverage

exceeded 50%. Nutrient analysis of seedlings treated with oil to cover 75-100% of the needle surface area show that the seedlings may have stressed and/or died as a result of the oil effects. The oil likely inhibited transpiration and/or photosynthesis. The effectiveness of ABG to remediate oil contaminated forest soils in greenhouse pots was demonstrated in this greenhouse study. When ABG was applied at rates of 1724 kg/ha, soil total petroleum hydrocarbon concentrations decreased from 18571 ppm to 574 ppm. Rates of ABG equal to or less than 431 kg/ha were not effective at remediating oil contaminated forest soils.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

For most of this century, Louisiana has provided the natural resources to support an extensive and successful petroleum industry. Louisiana is rich in oil reserves from both offshore sites in the Gulf of Mexico and onshore oil wells in the northern and central areas of the state. But with continued growth of the industry, there must also be an increase in awareness about protection of natural resources. Increased petroleum exploration and production increases the potential hazards to ecologically sensitive areas, such as wetlands and forests.

Louisiana contains over 30% of the nation's wetlands (Dahl, 1990). The only national forest in Louisiana, Kisatchie National Forest, covers much of the northern and central regions of the state. Oil production and exploration take place in both of these environments, and as a result, they are continually at risk to petroleum contamination due to accidental spills, leaks, or discharges. In 1997, 3780 oil spills were reported to the Louisiana Oil Spill Coordinator's Office (Davis, 1998).

Offshore oil spills are more common than onshore spills. Consequently, extensive research and development has been funded to prevent and cleanup these spills (Quina *et al.*, 1987). This is not the case for onshore spills. Remediation practices have been developed for onshore spills, but they are not always applicable for all onshore sites. Remediation of inland contaminated sites may include incineration of the oil, landfarming the contaminated soil, burial, containment in surface impounds, or deep well injection (Breitenbeck and DeSilva, 1995). These practices are not always a viable option for remediation of contaminated wetlands or forests due to the limited access for equipment and the ecologically sensitive nature of these environments. Cleanup of coastal wetlands is a common procedure due to their close proximity to offshore spills as oil is washed ashore. Inland spills are less common, especially those that involve interactions with wetlands and forests. There is an inadequate amount of literature that describes the impacts and remediation practices of oil contamination in these inland areas and the few writings that do exist can only be found in the 'gray' literature (Davis, 1998). 'Gray' literature is that which has not been formally published in recognized scientific or academic publications.

As a result, determining the best and most beneficial remediation practices to employ to cleanup petroleum contaminated wetlands and forests is difficult. Spills in these environments offer the chance to develop and implement remediation practices that are best suited for these situations. One such opportunity occurred when an oil well blowout took place in Cravens, Louisiana in August 1997. Approximately 24-30 hectares (60-75 acres) of the Kisatchie National Forest were affected by this eight-day oil and brine spill, including a 1.7 hectare (4.3 acre) freshwater depressional wetland adjacent to the oil well. A result of this blowout was the death and stressing of numerous loblolly and longleaf pine trees in the upland vicinity of the well. The assumption was the death was a result of oil and brine drift onto the trees and soil, but the mechanism within the plant is not well known. All of the wetland vegetation was killed. Since a spill of this nature and magnitude has never been recorded in the literature, it offered the opportunity to study and develop a remediation plan to restore both the upland pine plantation and wetland.

1.2 Objectives

The general objective of this study was to develop and implement a remediation and restoration plan for the affected upland forest and wetland. The study had the following specific objectives:

1. To determine the cause of death of the loblolly and longleaf pines affected by the blowout using nutrient concentration analysis;
2. To develop an approved remediation and restoration plan in conjunction with the National Forest Service for the impacted wetland;
3. To develop recommendations for the restoration and management of the affected upland forest areas.

1.3 Oil Spill Remediation

Most inland oil spills use traditional methods to clean up and remediate a site. These methods include recovery of oil by skimming or absorbence and disposal of oil by incineration, land farming, burial in land fills, containment in surface impoundments, deep well injection, and solidification (Bartha and Bossart, 1984). In the case of oil spills in wetlands or forests, these techniques are not always applicable due to limited access to oil cleanup equipment and the ecological sensitivity of these areas. Therefore, alternative methods must be developed.

1.3.1 Microbial Degradation

The natural pathway for petroleum decontamination is through microbial action. Many fungi and bacteria can either completely or partially metabolize the hydrocarbons from petroleum oils and fractions. Many reports describe the activity and distribution of oil degrading microorganisms in aquatic habitats (Atlas, 1975) and in uncontaminated and contaminated soils (Davis, 1967 and Odu, 1978). However, natural biodegradation and volatilization of petroleum hydrocarbons may not occur quickly enough to prevent extensive environmental damage to contaminated wetlands. Generally, the rehabilitation of contaminated surfaces relies on promoting the *in situ* biodegradation of contaminant oil (Atlas, 1977; Atlas *et al.*, 1978; Westlake *et al.*, 1978; Atlas, 1981). The ability of microorganisms to change the chemical composition of oil and promote its degradation has been proven in previous studies and is gaining recognition as an effective remediation strategy (Marconi and Nicola 1977; Odu, 1978; Westlake *et al.*, 1978).

1.3.2 Environmental and Physiochemical Factors

Many environmental and physiochemical factors influence the degradation rate and extent of hydrocarbon removal from contaminated soils. These include properties such as soil pH, moisture, aeration, and nutrient status. Contaminant characteristics such as molecular structure and toxicity to microbes, and the ecology of the microbial populations present in the soil also effect hydrocarbon degradation (Huesemann, 1994). The physical state of the oil determines its availability to microbes, while the chemical properties determine susceptibility to attack (Foght and Westlake, 1987). Any of these environmental or physiochemical factors can, at any time, increase or decrease the rate of petroleum degradation.

1.3.2.1 Redox

Previous studies have shown that hydrocarbon degradation occurs faster under aerobic conditions than under anaerobic conditions (Jobson *et al.*, 1972; Ward and Brock, 1978; DeLaune *et al.*, 1980; Hambrick *et al.*, 1980). Although anaerobic oil degradation has been demonstrated in several systems, it is a slow process, and is probably negligible in the environment (Foght and Westlake, 1987). Even in aerated environments hydrocarbon removal may be negligible. In the natural environment these oily materials become hydrophobic and flocculate together excluding water, soluble nutrients, and oxygen. Only a fraction of the flocculated material offers an environment suitable for colonization and degradation by microorganisms. Using sediment slurries under controlled redox potentials and pH, DeLaune *et al.* (1980), Hambrick *et al.* (1980) and Ward *et al.* (1980), found that up to 15% of added hydrocarbons degraded at Eh 220 and pH 8.0. The rate of hydrocarbon degradation was significantly higher at more positive redox potentials. Studies using oil contaminated soils (Jobson *et al.*, 1972) consistently showed that the highest rate of oil degradation occurred when soil aeration was improved. Similarly, Atlas *et al.* (1978) showed hydrocarbon utilization was sharply reduced in soils when O_2 was depleted.

1.3.2.2 Soil Moisture

Soil moisture plays an important role in the microbial degradation process. While a dry soil may reduce bacterial growth and survival, oxygen transfer to bacteria can be limited in a flooded soil, resulting in reduced hydrocarbon removal rates (Huesemann, 1994). The optimum soil moisture range for microbial degradation activity is between 50 and 80% of the moisture content at field capacity (Bartha and Bossert, 1984).

1.3.2.3 Nutrient Status

Besides oxygen and water, microorganisms require nitrogen (N), phosphorus (P) and other inorganic nutrients for growth when using petroleum as a substrate because hydrocarbons typically contain small amounts of these essential elements (DeSilva, 1995). During the biodegradation process, microbes incorporate carbon from the contaminant together with inorganic N and P from the soil into their cell structures (Huesemann, 1994). Laboratory experiments have shown that addition of nutrient salts (KNO_3 and Na_2HPO_4) to broth media increases the biodegradation of the petroleum by 70% (Bartha and Atlas, 1977). Similarly, Odu (1978) reported the result of a laboratory study showing that addition of nutrient solutions to soils amended with light crude oil enhanced O_2 uptake and CO_2 evolution. Treatment of oil contaminated field site with urea and phosphate fertilizers led to a rapid increase in bacterial density followed by rapid disappearance of n-alkanes, isoprenoids, and a continued loss in weight of saturated hydrocarbons (Westlake *et al.*, 1978).

The stimulatory effect of mineral nutrients on biodegradation varies depending on the methods and quantities applied. According to Dibble and Bartha (1979), the highest biodegradation rate in culture appeared to be at a C:N ratio of 15:1 and a C:P ratio of 200:1. To achieve those ratios, application of 1400 kg N and 100 kg P per ha would be required on a field contaminated with 100 metric tons of hydrocarbon. However, Hunt *et al.* (1973) found that although addition of low amounts of NH_4NO_3 stimulated biodegradation, an increase of N levels greater than 100 mg N/kg actually depressed soil respiration, possibly because of NH_3 or NO_3 toxicity. The American Petroleum Institute recommends a C:N ratio of 160:1, indicating that approximately 500 kg N or 5000 kg of 10:5:5 fertilizer is needed for remediation of a one ha site contaminated with 100 metric tons of hydrocarbons. In general, the amount of inorganic N fertilizer to be added depends upon the soil contaminant concentrations, the expected biodegradation rate, the amount of soil organic N, and the rate which organic N recycles (mineralization vs. immobilization) internally (McGill *et al.*, 1981).

1.3.2.4 Soil pH

The optimum pH range for microbial degradation of hydrocarbons in soil is 6 to 8 (Foght and Westlake, 1987). For contaminated salt marsh sediments, Hambrick *et al.* (1980) found that higher degradation rates occurred at a pH 8.0 than at 6.5 or 5.0. It appears that oil degradation is favored under slightly alkaline conditions (Foght and Westlake, 1987).

1.3.2.5 Salinity

One factor complicating the biodegradation process in soils is salinity. Salinity reduces the metabolic activity of many microbes (Walker and Calwell, 1975). Oil spills near production sites often result in both oil and brine contamination of the soil. Oil field brine has an electrical conductivity (EC) of about 200 dS (deciSiemens) per meter, which is nearly four times the EC of seawater (Kinghorn, 1983). Microbial degradation of hydrocarbons in hypersaline water (50 to 400 dS m^{-1}) is relatively slow (Ward and Brock, 1978). In their study of hydrocarbon biodegradation in hypersaline environments, Ward and Brock (1978) showed that an increase in salinity results in a decrease of microbial metabolism. Rhykerd *et al.* (1995) found that bioremediation of motor oil in soil was reduced when salinity was increased. They concluded that removal of salts from oil and salt contaminated sites before initiating bioremediation may reduce the time required for bioremediation.

1.4 *In Situ* Burning of Oil Spills

Oil spilled in ecologically sensitive environments, such as wetlands, pose unique problems when deciding which cleanup techniques to use for restoration. Traditional cleanup methods include landfarming the contaminated soil, using dispersing agents or detergents, containment within surface impoundments, and burial. These methods are not always applicable to oil contaminated wetlands, as they may result in more damage to the wetland than the oil itself (Mendelssohn *et al.*, 1996). Therefore, alternative remediation methods must be developed that will have no long-term effects on the wetland environment and can be incorporated into wetland management practices. One technique that has received much interest recently is *in situ* burning.

1.4.1 Fire Management in Wetlands

Prescribed burning within wetland habitats has long been used as a management tool (Kirby *et al.*, 1988). Advantages of wetland prescribed burns includes retarding succession and allowing for a more diverse community; increasing the availability of seek bearing food plants to waterfowl; providing succulent young growth for browsing waterfowl and mammals; opening up areas for better trapper success; creating open water for water fowl; and removing dense stands of vegetation and attracting waterfowl (Mendelssohn *et al.*, 1996). In Louisiana, most of the studies investigating wetland-fire interactions have taken place in the coastal environments. Kirby *et al.* (1988) state that inland wetlands are severely understudied from the standpoint of their interaction with fire.

1.4.2 Burning Impacts

In situ burning of oil spills has been employed as an oil spill cleanup technique for many years. This technique is rarely used on marine spills because of widespread concern over atmospheric emissions and uncertainty about its impacts on human and environmental health (US EPA, 1999). Many states, though, use *in situ* burning for inland spills. Some advantages of *in situ* burning include: high elimination rate; reduction of petroleum compounds to primary combustion products of carbon dioxide (CO_2) and water; minimal environmental impact; and minimal cleanup (Hillard *et al.*, 1999). Some negative impacts of *in situ* burning include: disturbance and death of biota; toxic residual compounds; and loss of detrital material important to the food web (Lindau *et al.*, 1995).

In situ burning can have varying impacts on vegetation. Vegetative responses to fire are commonly measured using biomass, primary productivity, stem number, stem height, cover, community structure, and species richness, diversity, and composition (Mendelssohn *et al.*, 1996). Several studies have shown favorable long-term plant growth responses after *in situ* burning (Baker, 1973; Kiesling *et al.*, 1988; Metzger, 1995). In contrast, many studies have illustrated the negative long-term effects of *in situ* burning (Holt *et al.*, 1978; McCauley and Harrel, 1981; Zischke, 1993; Tunnel *et al.*, 1995). A review of literature performed by Mendelssohn *et al.* (1996) showed that most wetlands recover from fire within one to five years. Trees and shrubs in freshwater swamps, though, are often suppressed or killed by fire, allowing wet prairies of aquatic or emergent plants to become reestablished (Glasser, 1985).

1.4.3 Factors

Before a wetland is burned several factors must be considered. These include chemical composition of the oil; season; type of vegetative cover and composition; depth of water before and after the burn; and any wildlife or human effects resulting from the burn. Mendelssohn *et al.* (1996) states that *in situ* burning is the most effective and compatible with wetland ecology when: summer burns are avoided; the wetland is dominated by rhizomatous herbaceous species, not shrub or tree species; water covers the marsh surface, or at least the soil is saturated at the time of the burn; and no long-term increase in water level is expected after the burn.

1.5 Ammoniated Cellulolytic Materials for Oil Spill Remediation

Use of cellulolytic materials as absorbents is a common cleanup technology in aquatic systems. Enriching readily available cellulolytic waste materials with N or other nutrients to enhance *in situ* or off site degradation of contaminant petroleum has received surprisingly little attention. An extensive search of the scientific and U.S. Patent literature resulted in only a few reports on the effects of modifying cellulolytic waste materials for use with oil spills. A patented process using treated cellulolytic materials and inorganic fertilizers with paraffin to maintain buoyancy and reduce solubility of nutrients for remediation of oil contaminated seawater was developed by Marconi and Nicola (1977). Similarly, Ericsson and Hedblom (1971) patented a method of oil absorption at sea by using cellulolytic materials rendered hydrophobic by treatment with heat decomposed ammonium or amide salts of aliphatic or cycloaliphatic carboxylic acids and oil. No conclusive laboratory or field data demonstrating the efficacy of these materials for enhancing microbial degradation was found in the scientific or engineering literature.

An alternative method to remediate oil spills using ammoniated organic wastes was developed by Gary Breitenbeck of the Louisiana State University Agricultural Center. This process involves ammoniating organic wastes with anhydrous ammonia at high temperatures and pressure. Examples of organic wastes used include bagasse, filterpress cake, rice hulls, and kenaf. Cellulose fibers are a principle component of all of these wastes. These fibers have highly porous structures that are capable of absorbing large amount of water and oil (Breitenbeck and DeSilva, 1995 and Breitenbeck and Grace, 1997).

When these wastes are ammoniated under high temperature and pressure, their nitrogen (N) content is increased. This N is bound in slowly available organic forms that ensure a dependable N supply for oil-degrading microorganisms (Breitenbeck and DeSilva, 1995). "Accelerated degradation results because crude oil or sludge is absorbed into an environment where surface area is greatly increased and microorganisms are provided with water, oxygen and nutrients needed or degradation (Breitenbeck and DeSilva, 1995)." Ammoniated bagasse (ABG) was found to be the most effective of the organic wastes tested. Its effectiveness was increased when it was incorporated into the soil instead of surface applied. ABG was found to consistently reduce hydrocarbon concentrations in coastal sands and saline marsh soils when added at the rate of 2 g ABG per 25 g soil. The principle fate of spilled oil was humification (Breitenbeck and DeSilva, 1995).

Ammoniated organic wastes offer an inexpensive and effective means of remediating oil contaminated shorelines and wetlands. The ammoniated material is lightweight, can be stored for long periods, has an attractive appearance and pleasant odor, and can be applied safely with negligible risk to personnel. A complement to adding ammoniated organic wastes would be incinerating the oil contained within the wetland, thus reducing the amount that would require bioremediation. This would allow for successful removal of the oil and restoration efforts could focus on removal of the brine from the area. Gypsum may also be incorporated along with the ABG in order to reduce the effects of increased salt concentrations caused by the brine from oil well blowouts.

1.6 Effects of Oil on Vegetation

The effects of oil on plants have been studied since the early 1900's. The majority, if not all, of this research focused on the effects of oil on agricultural crops and coastal salt marsh communities. As a result there is little published literature on the effects of oil on forest communities and, in particular, pine trees. Some general observations, though, of the effects of oil on agricultural and salt marsh plants are applicable to pine trees.

"Oil pollution effects may vary according to the type and amount of oil involved, the degree of its weathering, the time of year, and the species and age of the plants concerned (Baker, 1970)." Some of the effects that have been observed include yellowing and death of leaves, reduction of seedlings, reduction or ceasing of growth rate, and death of the plant.

1.6.1 Entry of Oil into Plants

Cuille and Blanchet (1958) were able to conclude that there are three factors related to phytotoxicity of oils: 1) the properties of the oil; 2) the quantity applied; and 3) the environmental conditions. deOng (1927) was able to distinguish between rapid or acute injury caused by light oils, and slow or chronic injury caused by heavier oils. In general, the smaller the hydrocarbon molecule, the more toxic the oil is to plants because smaller molecules can more easily enter the plant (van Overbeek and Blondeau, 1954).

Once a spill has occurred, the oil must be able to penetrate and move within the plant in order to cause injury. The oil molecule enters the plant more easily through stomata or at the point of contact (Baker, 1970). Once inside the plant, the oil travels through intercellular spaces into the plant cells (Knight *et al.*, 1929). Within the cell, the oil damages the plasma membrane and cell sap leaks into intercellular spaces (Baker, 1970). This leakage of cell sap causes the leaf to darken and lose turgor (Currier, 1951). This process, ultimately, upsets the physiology of the plant leading to stress or death.

1.6.2 Transpiration and Respiration Effects

Oils have also been found to affect transpiration, respiration, and photosynthetic rates of plants. Transpiration rates were found to be consistently reduced due to physical interference on or in the leaf tissue (Knight *et al.*, 1929; Bartholomew, 1936). The effects of oil on respiration rates vary with each plant species. Rates may be increased or decreased depending on the tolerance of individual plant species. Plant species with increased respiration rates include parsnip (Helson and Minshall, 1956) and bean (Green and Johnson, 1931; Green, 1936). Plant species with reduced respiration rates include: mustard (Helson and Minshall, 1956) and *Citrus* (Wedding *et al.*, 1952). Respiration rates are reduced when oil interferes with gaseous exchange by blocking stomata and intercellular spaces (Baker, 1970). Oil has been found to consistently reduce the rate of photosynthesis, primarily by physical interference with gaseous exchange similar to respiration reduction.

1.6.3 Nutrient Effects

Nutrient deficiencies or excesses may also have contributed to the injury of the trees. In a similar oil well blowout site in Union Hill, LA, pine needles of affected trees were analyzed for nutrient concentrations of ammonium (NH_4^+), nitrate (NO_3^-), boron (B), barium (Ba), nickel (Ni), strontium (Sr), sodium (Na), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and silicon (Si) (Hudnall, 1997). These trees showed similar effects as those suffered by the pine trees at the Cravens blowout site. The chlorotic appearance of the needles at Union Hill suggested a reduced uptake of N due to the blowout. After analysis, it was concluded that the trees' capacity to utilize N was reduced causing the chlorotic appearance. Boron, Ba, Ni, and Sr were found to have increased concentrations in the pine needles as a result of their reduced solubility.

The elevated concentration of Na was obviously the result of the brine from the blowout. The pine needles accumulated Na, which may have contributed to the loss of turgor and caused the needles to assume a distorted shape. Increased concentrations of K may have resulted from physiological accumulations or from surface residuals from the brine. Potassium regulates the opening and closing of stomata. The lack of turgor in the pine needles may have been due to the stomata being forced open, which then allows excessive transpiration. This would also contribute to the wilting of the needles. Magnesium and P are required for photosynthesis and their elevated concentrations may have inhibited this metabolic function. Increased S concentrations may be due to the result of S deposition. The pine trees at the Union Hill site were found to have more than a three-fold decrease of Si. Pine trees use Si to keep needles rigid and brittle. The solubility of Si increases in the presence of Na, and the increased levels of Na may have caused loss of Si from the needles. This process would cause the needles to take on a wilted appearance.

1.7 Effects of Brine on Vegetation

When an oil well blowout occurs, most of the effort is geared toward cleaning up and removing the oil. In most blowouts, brine (saltwater) is also a concern because of its adverse effects on vegetation and, indirectly, wildlife. Few, if any studies, have investigated the effects of brine on vegetation. Most of the research on the effects of sodium chloride (NaCl) or salts on plants has had the objective of improving crop yields in saline habitats (Treacy, 1984). The effects of salts on trees have received much less attention. Studies have investigated the effects of coastal salt spray and flooding and road de-icing salts on trees. An even smaller amount of research has investigated the effects of brine on forest vegetation. In general, the effects of NaCl on plants can be combined when the effects of brine on vegetation are investigated.

1.7.1 Salt Effects and Symptoms

Sodium has not been considered an essential plant nutrient (Treacy, 1984). Some studies, though, have indicated that sodium may be required by some plants (Brownell and Wood, 1957 and Brownell, 1965). Lunt (1966) has hypothesized that sodium may be required for certain enzymes, photosynthesis, or increased CO_2 assimilation.

The mechanisms by which salts damage plant tissues are poorly understood (Allen *et al.*, 1994). It has been suggested that salt adversely affects plant growth in two ways. First, high concentrations of specific ions in salt can be toxic and induce physiological disorders (Treacy, 1984). Second, soluble salts may lower the water potential in soil and restrict water uptake by the roots leading to drought or nutrient stress (Bernstein and Hayward, 1958).

The most common salt effect is a general stunting of plant growth (Treacy, 1984). Salt affected plants may be stunted, have darker foliage, and may have thicker and more succulent leaves (Jennings, 1976). A common visual effect of salt stress is leaf burn. Bernstein and Hayward (1958) describe leaf burn as tan or brown necrotic lesions sharply delineated from adjacent healthy green tissue, while cells adjacent to necrotic areas show no symptoms of deterioration. It is unknown what specifically causes leaf burn or why it occurs at leaf tips and margins, but both toxic and osmotic effects have been mentioned (Bernstein, 1975). In their studies on the effects of aerial drift from de-icer salts on trees, Lumis *et al.* (1973) found the conifer injury symptoms appeared as necrosis of the needle tip and the necrosis proceeded basipetally. In deciduous trees, injury symptoms appeared as twig dieback caused by the failure of vegetative and flower buds to develop (Treacy, 1984). In several oil well blowout sites, needle burn was observed on pine trees surrounding the sites.

Aerial salts, such as brine spray from an oil well blowout, may injure a plant by either foliar absorption or by soil salinization. Bernstein (1975) stated that rates of salt absorption by foliage are often 100 times that of roots. Many studies investigated the effects of road de-icing salts on vegetation adjacent to the roadway. The results have shown that their effects are primarily caused by foliar deposition, not soil salinization (Potts, 1978). Some of the effects are necrosis of leaf margins, stunted development, dieback of young shoots and leaves, and dieback of bark in some cases (Lumis, 1975). Feder (1977) found that saline mists generated by cooling at a power station damaged trees up to six miles away. Injury symptoms included: damage to dormant terminal buds, necrotic spots, burning and fall of leaves, delay in leafing, and petiole damage.

Mechanisms of salt resistance are still being elucidated (Greenway and Munns, 1980). The mechanisms of salt resistance fall into two broad classes: avoidance and tissue tolerance (Allen *et al.*, 1994). Salt resistance mechanisms among plants vary with species, variety, age, and environmental conditions (Mass and Hoffman, 1977). Salt resistance should not be measured by growth alone (Treacy, 1984). Leaf drop, leaf burn, and overall appearance should also be considered (Francois and Clark, 1978).

1.7.2 Brine

The primary byproduct of the oil drilling and production processes is brine. Brine fluids have high salt concentrations, usually twice that of seawater. Brine is usually stored in impoundment units during the separation process and then disposed by a number of ways. These include: injection into deep wells; sprayed on roads for dust control; treated and discharged to sewage treatment plants; or hauled to approved disposal sites (Walters and Auchmoody, 1989). Brine spills and accidental discharges pose serious environmental threats. However, the published literature does not adequately address impacts of brine on forest vegetation, its residual phytotoxicity, or vegetative recolonization patterns on brine killed areas (Auchmoody and Walters, 1988).

1.8 Oil and Brine Spill Restoration Case Studies

In the case of an oil and brine spill in Sam Houston National Forest, San Jacinto County, TX, two atypical methods were selected to mitigate the spill. These were freshwater flushing in areas principally affected by brine and controlled burning in oil-saturated areas (Zehner and Mullins, 1987). Approximately 12.5 acres of the forest and an unnamed tributary near the oil well were affected by the spill. In the area predominately contaminated by brine, freshwater was discharged in the attempt to dilute the standing or trapped brine, preventing the brine from saturating the forest soil. By increasing the fluid flow into the drainage pattern, it was expected that the oil in the zone would also be mobilized. During this time approximately 300,000 gallons of freshwater was flushed into the zone for 2.5 days. This reduced the chloride concentration to 1800 ppm, which is well below the 3000 ppm limit set by the Texas Railroad Commission. Based on these results it was concluded that this practice was successful.

In the area heavily affected by oil, it was decided that a controlled burn would be the best option to remove the oil. The burn appeared to drastically reduce the amount of oil on the surface and subsurface. The recommended restoration of the site after burning the oil and removing the brine included four parts: 1) clear cut the areas to remove all salvageable timber and expose the soil surface; 2) add 4 to 6 tons of lime per acre in order to increase the pH and enhance the natural populations of saprophytic fungi and bacteria; 3) reseed the area after four months with a mixture of endemic and weed species of grasses; and 4) reestablish pine trees after six to twelve months (Zehner and Mullins, 1987). The restoration techniques employed were deemed successful for this site.

In 1986, an oil spill along the Atlantic coast of Panama resulted in the death of approximately 75 ha of mangrove, primarily *Rhizophora mangle* (Teas *et al.*, 1989). Experiments were performed to determine the most beneficial method to reestablish the mangrove forest in soil that contained residual oil. The experiments tested the value of planting mangrove seedlings or propagules so that their roots could grow in non-oiled soil and possibly reach deeper, unoiled soil without contacting oil (Teas *et al.*, 1989). The most effective seedling protection was achieved by planting them in holes (25-35 deep and 25-35 cm in diameter) that were lined with plastic and backfilled with upland soil. Planting propagules deep in oiled soil, so that roots would be in a sub-surface low oil concentration zone, was ineffective as a restoration technique due to high mortality and poor growth.

Auchmoody and Walters (1988) performed a study investigating the effects of brine contamination in the Pennsylvania Allegheny National Forest.

Brine seeped from an impoundment and infiltrated the soil, killing all vegetation within a 1 ha (hectare) area. Mortality resulted exclusively from root-soil contact and did not involve foliar exposure. This was a spill of opportunity that allowed the authors to document the impact of brine on Allegheny hardwoods.

During the first growing season after the spill, herbaceous vegetation was eliminated and trees showed visual signs of stress. Hemlock was the first tree species affected. It had yellowish needles occurring acropetally, followed by browning, abscission, and finally death of the tree. Hardwood stress symptoms progressed from yellow leaves to premature leaf abscission resulting in sparse crowns, and then death.

After the brine was eliminated from the site, rapid establishment of a variety of annual and perennial herbaceous plants and commercial tree seedlings occurred. In following years, species diversity and coverage of herbaceous plants increased. The authors concluded that the rapid reestablishment of the area was evidence that residual phytotoxicity of brine is short lived. Minimal concentration of hydrocarbons at the site may have also contributed to rapid colonization (Auchmoody and Walters, 1988).

1.9 Wetland Restoration

Population expansion across the country has resulted in the draining and altering of wetlands to accommodate human needs. From the 1780's to the 1980's, wetland area in the United States decreased from 89.5 million ha to 42.2 million ha (Dahl, 1990). However, enactment of strong wetland protection laws in the mid-1970's has helped to slow the rate of loss. The public has only recently become aware of the many benefits that wetlands provide and realized the need for wetland protection regulations. Benefits of wetlands include water quality improvement, flood attenuation, aesthetics, recreation, sediment trapping, and erosion control. When wetlands are degraded or destroyed these valuable attributes are lost and often unreplaceable. The benefits of restoring degraded or destroyed wetlands and creation of new wetlands have only recently been recognized.

The science of restoration ecology, especially wetland restoration, is young and in continual development. As a result, there has been much confusion as to how the term wetland restoration should be defined in relation to similar terms, such as created wetlands, constructed wetlands, and enhanced wetlands. The Society of Wetland Scientists defines wetland restoration as, "Actions taken in a converted or degraded natural wetland that result in the reestablishment of ecological processes, functions, and biotic/abiotic linkages and lead to a persistent, resilient system integrated within its landscape" (Society of Wetland Scientists, 2000). Lewis (1990) defines wetland creation as the construction of a wetland on a site that never was a wetland. Enhanced wetlands are defined as an existing wetland that has been altered to improve a particular function, usually at the expense of other functions (Lewis, 1990). Constructed wetlands are wetlands that are developed with the primary purpose of contaminant or pollution removal from wastewater or runoff (Hammer, 1997).

1.9.1 Legal Protection of Wetlands

The primary vehicle for wetland protection and regulation in the United States is Section 404 of the Federal Water Pollution Control Act (FWPCA) amendments of 1972 (Mitsch and Gosselink, 2000). Section 404 requires that anyone dredging or filling in "waters of the United States" must request a permit from the U.S. Army Corps of Engineers. If a project has potential detrimental effects on wetlands, then three approaches are evaluated in sequence: 1) avoidance - taking steps to avoid wetland impacts where practicable; 2) minimization - minimizing potential impacts to wetlands; and 3) mitigation - providing compensation for any remaining, unavoidable impacts through the restoration or creation of wetlands (Mitsch and Gosselink, 2000). This has led to the practice of requiring that wetlands be created, restored, or enhanced to replace wetlands lost in development such as highway construction, coastal drainage and filling, or commercial development (Mitsch and Gosselink, 2000). These wetlands are termed mitigation wetlands.

Additional government programs, such as the USDA Conservation Reserve Program (CRP), Wetland Reserve Program (WRP), Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA), and Federal Agriculture Improvement and Reform Act, were created to enforce wetland protection and increase the practice of wetland restoration.

1.9.2 Success and Evaluation

These laws and regulations have forced wetland scientists to study and continually develop the art of wetland restoration. Attempts at creating and restoring specific types of wetlands, including tidal wetlands, mangroves, freshwater marshes, and forested wetlands, have had mixed results. Often goals for these projects are not well stated and information on the existing wetland functions is not well documented (Kentula, 1996). Monitoring during and after a project is completed is often uncommon. The monitoring reports that do exist are scarce, even in the gray literature (Clewell and Lea, 1990).

Often the focus of wetland creation or restoration projects is on the short-term success rather than long term sustainability. Defining success is one of the more difficult aspects of wetland restoration projects because there is no generally accepted definition of success and methods of evaluation have not been standardized (Kentula, 2000; D'Avanzo, 1990). These problems have been studied and documented in California (Josselyn and Buchholz, 1984; Eliot, 1985; Race, 1985) and Washington (Kunz *et al.*, 1988). D'Avanzo (1990) lists four reasons explaining the difficulty of evaluating the long term success of restoration projects, including: 1) wetlands are created or restored for a wide variety of purposes; 2) little is known about many basic aspects of ecosystem level processes in wetlands; 3) predicting vegetation change over time (succession) in many wetland types is difficult; and 4) wetlands are exceedingly varied, highly dynamic systems.

Kentula (2000) defines three types of success: 1) compliance success - determined by evaluating whether the project complies with the terms of an agreement; 2) functional success - determined by evaluating whether the ecological functions of the system have been restored; and 3) landscape success

- measure of how restoration has contributed to maintaining or improving the ecological integrity of the region or landscape. In theory, long term success of wetland restoration projects should incorporate all three classes. Criteria typically used in wetland ecosystem studies can be used to evaluate long term success. These criteria may include comparison of vegetation growth characteristics in artificial and natural wetlands after two or more growing seasons, habitat requirements of plants invading the created or restored site, success of planted species, and chemical analyses of artificial wetland soils compared to natural wetlands (D'Avanzo, 1990).

1.9.3 Reference Wetlands

One approach used by the Wetlands Research Program of the U.S. EPA to determine success is the use of reference wetlands. Reference wetlands are natural wetlands that serve as reference sites against which project wetlands are judged. These wetlands are often sites within a specific geographic region chosen to encompass the known variation of the group or class of wetlands of interest (Brinson and Rheinhardt, 1996). When using this technique the determination of success depends heavily on the choice of the reference site and on how well it embodies the goals of the restoration (Kentula, 2000).

Comparisons between the project wetland and reference wetland can include ecosystem functions, water quality, species diversity, and flora and faunal populations. Wetland functions that may be assessed include groundwater recharge, groundwater discharge, flood storage, sediment trapping, food chain support, wildlife habitat, and fishery habitat (Erwin, 1990). Reference wetlands are used more often for marsh creation or restoration projects than for forested wetlands because often functions of a forested wetland ecosystem require full forest development before they can be compared with reference wetlands (Clewell and Lea, 1990).

1.9.4 Restoring Hydrology and Vegetation

1.9.4.1 Hydrology

Wetlands are defined by hydrology, hydrophytic vegetation, and hydric soils. The success of wetland establishment centers around the hydrology of the system. Hydrology is the single most important factor to consider in designing and implementing restoration projects for specific types of wetland systems and their related functions (Erwin, 1990). Engineering activities that can be implemented to restore wetland hydrology include flashboard risers, flap gates, retention ponds, and spillways (Clewell and Lea, 1990). Other approaches to restore wetland hydrology include levee and embankment removal, artificial channel backfilling, and dechannelization. These are fairly new restoration approaches, and little research exists to document their success (Middleton, 1999). Restoration projects using some of these techniques have taken place in Florida (Shirley, 1992 and Toth, 1996), Louisiana (Turner *et al.*, 1988; Trepagnier *et al.*, 1995), California (Haltiner *et al.*, 1997), Oregon (House, 1996), and France (Bravard *et al.*, 1986).

1.9.4.2 Revegetation

Reestablishment of desirable vegetation after hydrology has been reestablished is an essential, but sometimes difficult, step in wetland restoration.

The species of vegetation types to be introduced to restored wetlands depend on the type of wetland desired, the region, and the climate (Mitsch and Gosselink, 2000). Species that should be introduced are those that are indigenous and typical of mature, undisturbed, local stands of the community being restored (Clewell and Lea, 1990). A plant species list developed from local reference wetlands can be used as a guideline for appropriate vegetation to be established.

In freshwater marshes, common vegetation for restoration may include bulrush (*Scirpus* spp.), cattail (*Typha* spp.), and sedges (*Carex* spp.). Coastal marsh vegetation includes smooth cordgrass (*Spartina alterniflora*) and common reed (*Phragmintes australis*). Forested wetland restoration usually involves the establishment of seedlings. Species may include willow oak (*Quercus phellos*), water oak (*Q. nigra*), nuttall oak (*Q. nuttallii*), green ash (*Fraxinus pennsylvanica*), and sweetgum (*Liquidambar styracifula*). Deepwater vegetation such as bald cypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*) are used less frequently.

When restoring wetland vegetation, a concerted effort should be made to prevent establishment of noxious, invasive, and alien plant species. Rejmanek and Richardson (1996) define invasive species as those that spontaneously (and aggressively) spread after deliberate or inadvertent introduction to a new locale. When these species become established on a restored site they can out-compete and displace native species, reduce wildlife habitat potential, alter natural ecosystem processes, and limit overall biodiversity (Melvin, 1999). These invasive species succeed because they are good colonizers and competitors and are difficult to remove once established (Galatowitsch *et al.*, 1999). Examples of invasive species include Chinese tallow (*Sapium sebiferum*), hybrid cattail (*Typha glauca*), paper bark tree (*Melaleuca quinquenervia*), purple loosestrife (*Lythrum salicaria*), and reed canary grass (*Phalaris arundinacea*).

Studies by Reinartz and Warne (1993) and Mitsch *et al.* (1998) found that the way vegetation is established can affect the diversity and success of the restored wetland system. When revegetating a restoration site, a decision must be made to determine if the site will be naturally revegetated or if artificial means will be incorporated. Natural revegetation mimics natural wetland successional processes. This is an inexpensive process, but is site dependent. In order for this method to succeed, the hydrology of the project site must be accurately restored and a healthy seed bank must be present. Using this technique may cause an initial period of invasion by undesirable species, but if proper hydrologic conditions are imposed, these invasions should be temporary (Mitsch and Gosselink, 2000). When used successfully, natural revegetation ensures that there will not be an artificial quality to the composition or spatial configuration of the vegetation (Middleton, 1999). Farmer *et al.* (1982), Rushton (1983), and Wade (1986) have reported restoration projects where natural revegetation was successful.

When a project site does not have adequate numbers or abundance of desired species, artificial revegetation may need to be utilized (Davis, 1995). This technique involves human intervention to introduce desired species to a site. Options for artificial revegetation include planting of seeds, bare root seedlings, containerized seedlings, stem cuttings, rhizome cuttings, and plant-soil transfers (Clewell and Lea, 1990; Davis, 1995; Mitsch and Gosselink, 2000). One technique of artificial revegetation that has drawn considerable interest over the past decade is the use of donor wetland soil. Donor wetland soils may contain between 2000 and 50,000 seeds per square meter. This usually results in a diverse seed bank containing viable seeds. Donor wetland soils may also increase water retention capability and introduce microorganisms and fungi to a restored wetland (Clewell and Lea, 1990). Studies by Burke (1997) and Brown and Bedford (1997) have reported successful restoration of wetlands using this technique.

The two basic sources of wetland vegetation planting materials are commercially grown plant material and native plant material collected from naturally occurring populations (Davis, 1995). There are advantages to both sources of vegetation. In general there is more control over the timing, quality, and quantity from commercially available plant material than from native sources (Davis, 1995). Native plant sources are more likely to provide a suite of species similar to reference wetlands and diverse genetic stock that is adapted to local conditions (Davis, 1995). Whatever type revegetation technique is used, it is important to understand the wetland in relation to its surrounding landscape and choose the method that is best for that particular site. The persistence of obligate wetland plants with time – either planted or naturally colonizing – and their successful dominance over other vegetation, is one good measure of restoration failure or success (D'Avanzo, 1990).

CHAPTER 2

REMEDIATION OF AN OIL CONTAMINATED WETLAND

2.1 Introduction

Oil spills are common occurrences in the oil exploration and production industry. Brown (1987) estimated that 6,000,000 tons per year of oily wastes enter the environment uncontrolled. In 1997, 3780 oil spills were reported to the Louisiana Oil Spill Coordinator's Office (Davis, 1998). Most of these are marine spills that usually amount to less than seven tons of oil per incident. Nevertheless there is an increase in the frequency of oil spills in terrestrial environments. With this increase of terrestrial incidents, it has become necessary to develop remediation strategies that will effectively remove the oil with the least amount of harm to the environment. Cleanup methods that are often employed in terrestrial spills include landfarming the contaminated soil, burial, containment in surface impounds, or deep well injection (Breitenbeck and DeSilva, 1995). These methods are not always a viable option when remediating oil spills in ecologically sensitive environments, such as wetlands. Traditional remediation procedures employed in these environments may result in more damage to the area than from the oil itself.

Bioremediation is a practice that is commonly employed to cleanup oil spills in terrestrial environments. Microbial degradation of petroleum products in soil, either via naturally occurring or facilitated methods, is a process that is successfully used to reduce soil concentrations of the contaminant to acceptable levels (Englert *et al.*, 1993). During this process, the microbes attack only specific types of hydrocarbons rather than the entire oily waste. As a result, hydrocarbons are converted to carbon dioxide, water, biomass, or humic materials (Huesemann, 1994). This is a preferred method to use in oil contaminated wetlands because it results in less harm to the environment than traditional techniques.

Enhancing the bioremediation process can help speed the recovery of the oil contaminated area. This can be accomplished using fertilizers or ammoniated organic wastes, such as bagasse. Bagasse is the organic material remaining after the juice has been squeezed from sugarcane stalks. Cellulose is a primary component of bagasse and it is these cellulose fibers that are capable of absorbing large amounts of oil and water. When bagasse is applied to the surface of contaminated soils it wicks oil from the subsurface to the upper soil depths. Increasing the nitrogen content of bagasse promotes the microbial decomposition of oil by providing an environment that contains oxygen, water, and nutrients, all essential elements needed for successful microbial remediation. Previous studies using ammoniated bagasse have resulted in successful removal of hydrocarbons in coastal marsh soils (DeSilva, 1995 and Grace, 1997).

The objective of this study was to assess the effectiveness of ammoniated bagasse to remediate a wetland contaminated with oil and brine following an oil well blowout. This spill took place in Cravens, Louisiana in August of 1997. Approximately 24-30 hectares (60-75 acres) of the Kisatchie National Forest were affected by this oil and brine spill, including a 1.7 hectare (4.3 acre) freshwater depressional wetland adjacent to the oil well. All of the wetland vegetation was killed and the soil was severely contaminated by oil and brine. Since a spill of this nature and magnitude has never been recorded in the literature as to date, it offered the opportunity to study and develop a remediation plan that would lead to the restoration of the wetland.

2.2 Materials and Methods

2.2.1 Description of Study Area

The study area is located in Cravens, LA (30°59' N, 93°01' W) within the Kisatchie National Forest (Figure 2.1). The impacted freshwater depressional wetland lies approximately 0.2 kilometers (1/8 mile) south of the oil well. After the oil well blowout, an earthen berm was constructed in order to protect the wetland from any overland oil runoff. The berm failed after a heavy rain washed it away, and as a result oil and brine flowed unimpeded into the wetland (Figure 2.2). Overland flow of oil also occurred west of the well into Little Sixmile Creek, which is located less than 1/8 mile from the well.

The soils of the upland area have been mapped as a Ruston fine sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Paleudult). The soil of the wetland is a Guyton silt loam (Fine-silty, siliceous, active, thermic Typic Glossaqualf), a typical wetland soil. Loblolly pine (*Pinus taeda*) and longleaf pine (*Pinus palustris*) dominate the vegetation of the upland area. Dominant vegetation within the wetland includes sweetgum (*Liquidambar styraciflua*), black gum (*Nyssa sylvatica*), and tupelo gum (*Nyssa aquatica*).

2.2.2 C-K Associates, Inc. Preliminary Site Data

After the oil well blowout in August of 1997, C-K Associates, Inc. was hired by the owners of the well to conduct an ecological and environmental assessment of the site. A sample of oil from the wellhead was characterized for petroleum

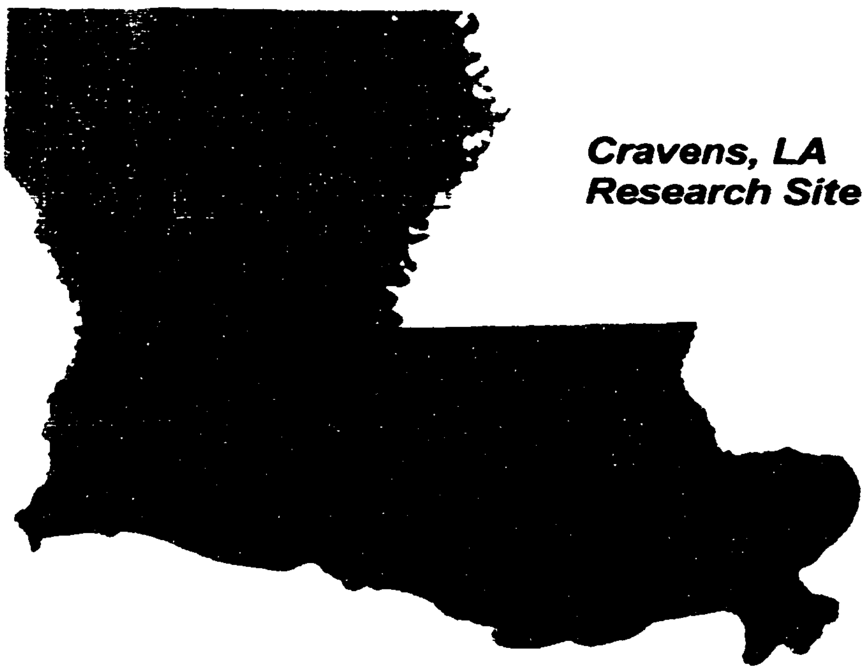

Figure 2.1 Location of Cravens, LA Oil Contaminated Wetland.

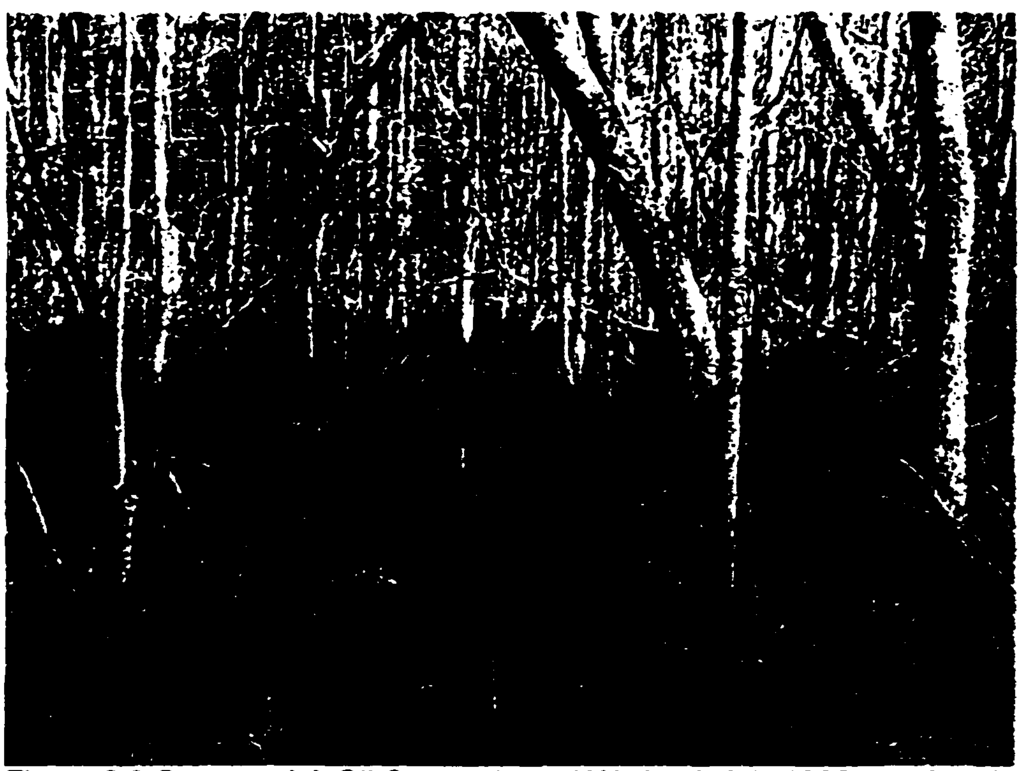

Figure 2.2 Cravens, LA Oil Contaminated Wetland, July 1998 (pre-burn).

hydrocarbons (Table 2.1). The total petroleum hydrocarbon (TPH) concentration was 910,000 ppm and the concentrations of 2-methylnaphthalene and naphthalene were the highest of the semi-volatile organic compounds. In June of 1998, C-K Associates, Inc. collected wetland soil samples from three random locations within the wetland and analyzed these samples for TPH, electrical conductivity, pH, and priority pollutant metals (Table 2.2). The elevated concentrations of TPH revealed the need for oil remediation of the contaminated wetland soil.

2.2.3 Wetland Remediation

The oil well blowout occurred during August 1997. During July 1998, soil samples from four randomly selected locations within the wetland were taken at depths of 0-10, 10-20, and 20-30 centimeters (cm). The electrical conductivity (EC) and pH for the soil samples were determined using a saturated soil paste extract according to the methods of Page *et al.* (1982). The samples were analyzed for EC in order to determine the extent of brine contamination within the wetland. After much discussion, the United States Forest Service decided the most economic and efficient method for removing the surface oil from the wetland was incineration.

The wetland was burned in December 1998 (Figure 2.3). The burn resulted in volatilization of nearly all of the surface oil (69,000 ppm to 2000 ppm). Three days after the burn, three transects were established within the wetland and five locations, at 10 m intervals, along each transect were selected for soil sampling. Sampling depths were 0-10, 10-20, and 20-30 cm. These samples were analyzed for pH, EC, and sodium according to the methods of Page *et al.* (1982). Soil samples from four selected upland sites were also taken at the ends of two of the

Table 2.1 Petroleum Hydrocarbon Characterization for Oil Wellhead Sample from Cravens, Louisiana Oilwell Blowout Site.*

Semi-volatile Organic Compound	Concentration (ppm)
2-methylnaphthalene	2100
Acenaphthalene	<198
Acenaphthylene	<198
Anthracene	<198
Benzo(a)anthracene	<198
Benzo(a)pyrene	<198
Benzo(b)fluorathene	<198
Benzo(h,h,I)perlyene	<198
Benzo(k)fluorathene	<198
Chrysene	<198
Dibenz(a,h)anthracene	<198
Fluorathene	<198
Fluorene	<198
Ideno(1,2,3-c,d)pyrene	<198
Naphthalene	200
Phenanthrene	<198
Pyrene	<198

*From C-K Associates, Inc. (1999)

Table 2.2 Soil Data from Oil Contaminated Wetland at Cravens, Louisiana Oilwell Blowout Site (June 1998).*

TPH – 69,000 ppm

Electrical Conductivity – 837 μS/m

pH – 5.05

Priority Pollutant Metals

Metal	Concentration (ppm)
Antimony	<1.0
Arsenic	<1.0
Beryllium	<0.2
Cadmium	<0.5
Chromium	2.1
Copper	1.3
Lead	6.7
Mercury	<0.1
Nickel	<1.0
Selenium	<0.5
Silver	<1.0
Thallium	<1.0
Zinc	1.6

*From C-K Associates, Inc. (1999)


Figure 2.3 Cravens, LA Oil Contaminated Wetland, December 1998 (post-burn).

transects. These samples were taken at depths of 0-10, 10-20, and 20-30 cm and analyzed for pH, EC, and sodium (Na) concentration according to the methods of Page *et al.* (1982).

Ammoniated bagasse (ABG) was used in combination with lime ($CaCO_3$) and topsoil to test the effectiveness of the bagasse to bioremediate the wetland. Test plots were established using open-ended cylinders (30 cm diameter / 105 cm height) constructed from plastic corrugated drainage pipe driven 15-20 cm into the soil (Figure 2.4). In May 1999, the bagasse, lime, and topsoil were added to each cylinder. One week before the bagasse was added, $CaCO_3$ was incorporated into

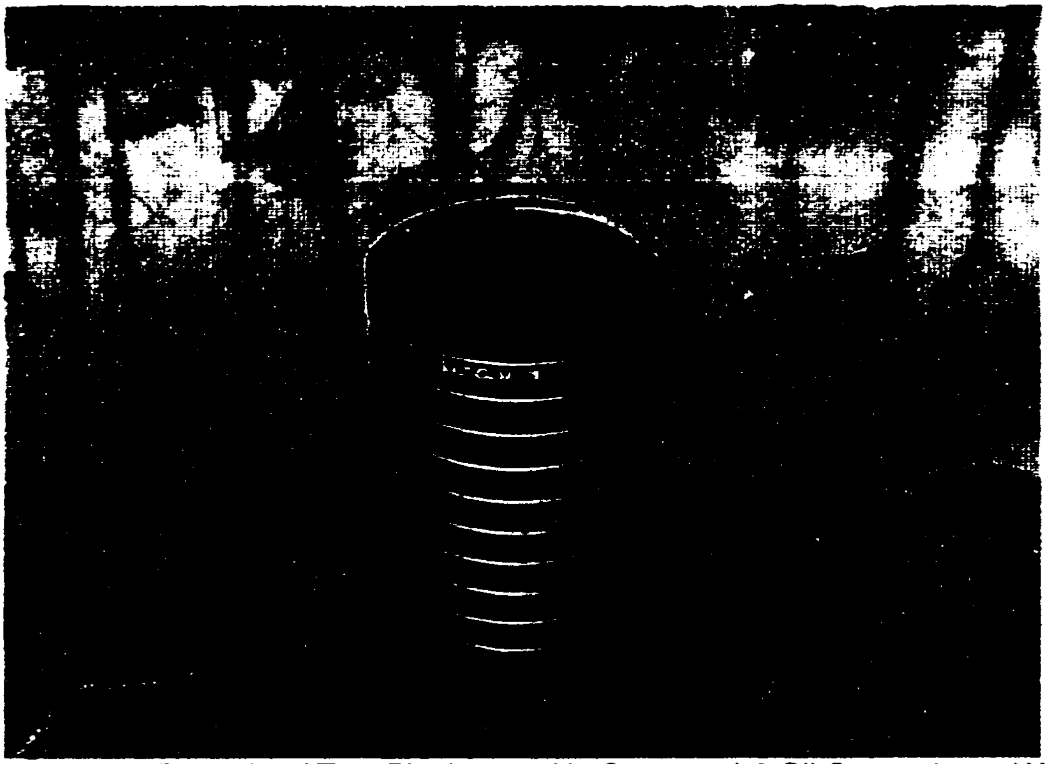

Figure 2.4 Example of Test Plot Located in Cravens, LA Oil Contaminated Wetland.

the top 5 cm of the soil at a rate of 1500 kg/ha in order to raise the pH of the soil to approximately 6.5. The rates of bagasse added were 0, 50, 100, and 200 kg/ha, with five replications of each treatment randomly placed within the wetland. These rates were based upon the post-burn total petroleum hydrocarbon (TPH) analyses performed by C-K Associates, Inc. (1999). Topsoil collected from the non-contaminated upland area was added to the wetland soil at a rate of 0.015 kg/ha in order to ensure an adequate microbial population within each cylinder. The bagasse and topsoil were mixed together and incorporated into the top 2 cm of the soil.

Soil samples from each cylinder were collected at depths of 0-5, 5-10, and 10-20 cm at 21 day intervals beginning in June 1999 and analyzed for TPH, EC, and pH. After a 90 day study period the test cylinders were removed and soil samples were collected at depths of 0-10 and 10-20 cm.

Total petroleum hydrocarbon concentration for all samples was determined using a Buck Scientific Total Hydrocarbon FT-IR Analyzer using a known concentration of weathered crude oil obtained from the research site. Residual oil concentration for all soil samples collected was determined in aliquots of mixed soil extractions using carbon disulfide (CS_2) as the extractant. CS_2 was used because hydrocarbon compounds are readily miscible in it and it does not contain any C-H bonds that would interfere with FT-IR analysis (Barre, 1997). A standard curve was created using weathered crude oil taken from the contaminated wetland. Standard solutions of 10, 25, 50, 75, and 100 ppm were used to calibrate the hydrocarbon analyzer each day before analysis.

Fifteen grams of contaminated wetland soil was placed into a glass jar. Twenty milliliters of CS_2 was added to the soil and the jar loosely sealed. The soil and CS_2 were sonified for 30 minutes. One milliliter of the CS_2 extractant was collected after sonification using a glass syringe and diluted with non-contaminated CS_2. This extractant sample was then analyzed using the Buck analyzer and TPH concentration determined from the standard curves created earlier.

Electrical conductivity and pH were analyzed according to the methods of Page *et al.* (1982). A Perkin-Elmer ICP-AES was used to analyze the soil samples taken after the 90 day study period for water-soluble cations (Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, and Zn). Statistical analysis of the wetland soil hydrocarbon data was performed using the SAS GLM procedure. An α-value of 0.05 was used to determine the significance of interactions of treatment, date, depth, and replications.

2.3 Results

2.3.1 Pre- and Post-burn Soil Analysis

Pre-burn wetland soil analysis from samples collected in July 1998 (Table 2.3) indicated that the 0-10 cm soil layer had a low concentration of brine with an average EC of 434 µS/m (microSiemens per meter). The average EC readings for the 10-20 cm and 20-30 cm layers were 637 µS/m and 611 µS/m, respectively. pH readings for the soil ranged from 4.7 to 5.5.

Post-burn analysis of the wetland soils sampled in December 1998 showed a decrease in EC (Table 2.4). The average electrical conductivity of the 0-10 cm soil layer was 236.7 µS/m, average pH was 5.2, and the average Na concentration was 26.6 ppm. The average EC for the 10-20 cm soil layer was 259.7 µS/m, average pH was 4.8, and average Na concentration was 29.7 ppm. The average EC for the 20-30 cm soil layer was 374.9 µS/m, average pH was 4.6, and average Na concentration was 31.5 ppm.

Comparing EC results for the pre- and post-burn soil analysis indicated a decrease in salinity after the burn. In the 0-10 cm soil layer the EC decreased from 434 µS/m to 236 µS/m, in the 10-20 cm layer from 637 µS/m to 259 µS/m, and in the 20-30 cm layer from 611 µS/m to 374 µS/m. The differing pH values are normal for wetland soils. As a result of these analyses, the US Forest Service concluded

Table 2.3 Pre-Burn Wetland Soil Analysis from the Cravens, LA Oilwell Blowout Site (July 1998).*

Sample Depth	Electrical Conductivity (s.d.) (μS/m)	pH (s.d.)
0-10 cm	434 (130)	5.5 (0.3)
10-20 cm	637 (273)	4.9 (0.8)
20-30 cm	611 (281)	4.7 (0.6)

Table 2.4 Post-Burn Wetland and Upland Soil Analysis from the Cravens, LA Oilwell Blowout Site (December 1998).*

Sample Location And Depth	Electrical Conductivity (s.d.) (μS/m)	pH (s.d.)	Sodium (s.d.) (ppm)
Wetland 0-10 cm	236.7 (115.9)	5.2 (0.9)	26.6 (13.9)
Wetland 10-20 cm	259.7 (102.9)	4.8 (0.6)	29.7 (15.6)
Wetland 20-30 cm	374.9 (145.2)	4.6 (0.5)	31.5 (21.6)
Upland 0-10 cm	262.8 (37.5)	5.4 (0.9)	20.3 (6.5)
Upland 10-20 cm	175.3 (55.0)	5.0 (0.7)	16.5 (8.0)
Upland 20-30 cm	167.0 (47.5)	5.0 (0.6)	14.5 (10.4)

that the brine was no longer a deterrent to wetland remediation and restoration because the soil salinities were well below the critical value (400,000 μS/m) necessary for vegetative survival (Bohn *et al.*, 1985). Efforts then focused on removal of the residual oil in the soil.

Post-burn analysis of the upland soil samples is also presented in Table 2.4. As expected, these soils had lower electrical conductivities, due to less brine contamination, and higher pH values when compared to the wetland soils. As with the wetland soils, brine will not be an obstacle to overcome when restoring and managing vegetation on these upland environments.

2.3.2 Soil Hydrocarbon Analysis

The 90-day period for the wetland study ended in September 1999. Soil samples taken in June, July, August, and September were analyzed for TPH. Results of the analyses suggest that the ABG decreased the overall hydrocarbon concentration of the contaminated wetland soils. The mean hydrocarbon concentration for all soil samples collected during June, July, August, and September was 626 parts per million (ppm), with a maximum concentration of 9643 ppm and a minimum concentration of 5 ppm. This is decrease in the post-burn TPH concentration of 2000 ppm measured by C-K Associates, Inc. (1999). The standard deviation of 1347 ppm illustrates the high variability of oil concentration within the wetland.

The results of the TPH soil analyses are presented in Tables 2.5 and 2.6 and Figures 2.5 and 2.6. Replications of each treatment have been averaged. Since there was no significant difference in the TPH values (p=0.8592) of the 0-5 cm and 5-10 cm layers in June, July, and August, these values were averaged into one composite 0-10 cm layer in order to compare results with the last sampling period.

In the 0-10 cm layer (Figure 2.5), the wicking action of ABG, which has been proven in previous studies with ABG (DeSilva, 1995 and Grace, 1997), may also be occurring in the 100 and 200 kg/ha treatments in this study. In June, 21 days after the bagasse was added, the 200 kg/ha treatment had the highest mean concentration of oil (904 ppm) compared to the other treatments, as the bagasse may have drawn oil from the subsurface to the soil surface. From June to July, the 200 kg/ha treatment may have continued to wick oil to the surface, which would be represented by the increase in mean TPH concentration (904 ppm to 942 ppm). From July to August there is a decrease (942 ppm to 199 ppm) in mean TPH in the 200 kg/ha treatment. Previous studies (DeSilva, 1995 and Grace, 1997) have shown that as oil is absorbed by the bagasse, accelerated microbial degradation decreases TPH concentration. Similar accelerated degradation may have occurred in the Cravens wetland soils and would account for the decrease in the 200 kg/ha treatment from July to August. The increase in mean TPH from August to September in the 200 kg/ha treatment (199 ppm to 1336 ppm) may be as result of the microbes exhausting their nitrogen source (the ABG) or the occurrence of a substantial rain event in August 1999. As a result of this rain event, residual oil that remained in the 10-20 cm layer may have been translocated to the 0-10 cm layer, therefore increasing the TPH concentration in the surface soil.

As a result of high spatial variability of oil within the wetland, statistical analysis of the 200 kg/ha treatment shows no significant difference in TPH concentration throughout the entire 90 day study period (Table 2.5). This could lead to the conclusion that the ABG had no effect on the decrease of TPH

Table 2.5 Total Petroleum Hydrocarbon Means ($\bar{x}$) and Standard Deviations (s) for 0-10 cm Soil Layer in the Oil Contaminated Wetland at Cravens, LA as a Result of Different Ammoniated Bagasse (ABG) Treatments.

ABG Treatment Applications	June		July		August		September	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
0 kg/ha	215^{Aa}	99	335^{Aa}	280	402^{Aa}	425	2960^{Bb}	2775
50 kg/ha	164^{Aa}	89	290^{Aa}	296	422^{Aa}	603	371^{Aa}	155
100 kg/ha	747^{Aab}	715	242^{Ab}	143	246^{Ab}	194	2071^{Ba}	1598
200 kg/ha	904^{Aa}	932	942^{Aa}	811	199^{Aa}	237	1336^{Ba}	1080

Means with the same lower case letters within rows are not significantly different. Means with the same upper case letters within columns are not significantly different.

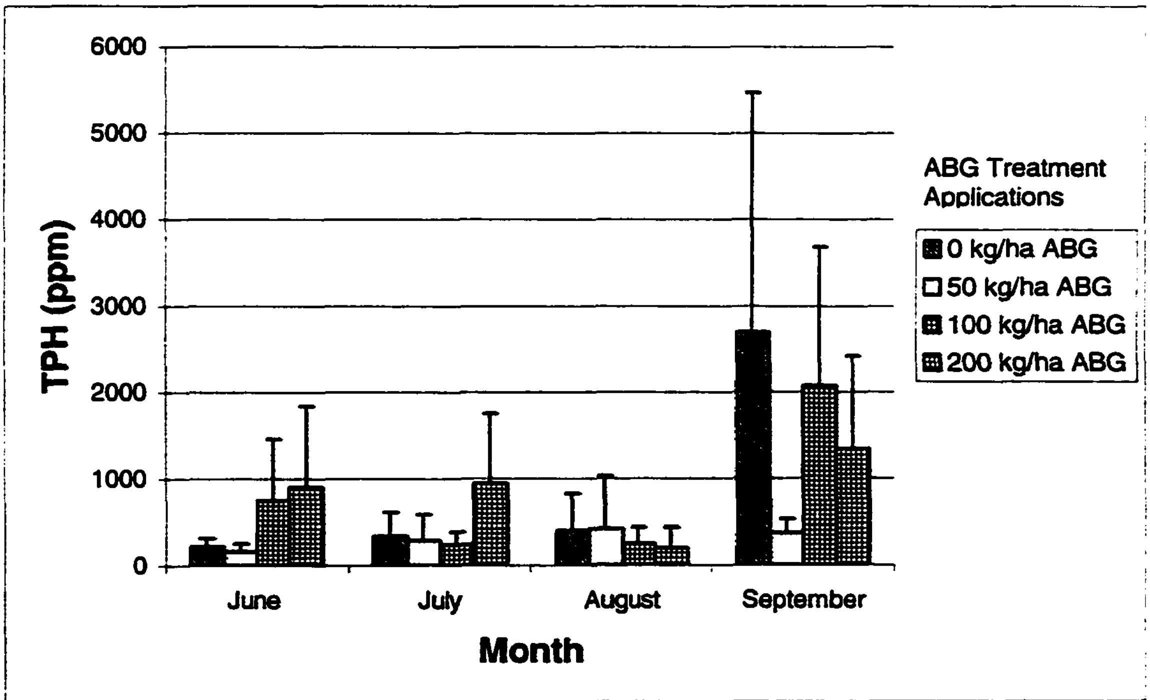

Figure 2.5 Total Petroleum Hydrocarbons in the 0-10 cm Soil Layer from the Oil Contaminated Wetland at Cravens, LA Resulting from Different Ammoniated Bagasse (ABG) Treatments (error bars are s.d.).

concentrations in the soil. This variability precludes determining the ability of the ABG to bioremediate the wetland.

The 100 kg/ha treatment shows a similar trend as the 200 kg/ha treatment during the study period. The 100 kg/ha treatment seems to be wicking oil from the subsurface to the soil surface from in June, but there is no statistical difference between the other treatments during this period to support this conclusion. As a result, the 100 kg/ha treatment may not be wicking oil to the surface. There seems to be a decrease in mean TPH concentration from June to July in the 100 kg/ha treatment (747 ppm to 242 ppm), but there is no statistical difference between the June and July data to positively prove this. The increase in mean TPH concentration from August to September (246 ppm to 2071 ppm) seems to be as a result of the rain event that occurred in August. Oil may have been moved from the subsurface to the surface as water moved through the wetland. The statistical difference between the August and September data would help to support this conclusion.

The 50 kg/ha treatment had little or no effect on the degradation of oil in the wetland soils. The TPH values for the 50 kg/ha treatment ranged from 164 ppm to 422 ppm. There is no statistical difference in TPH concentration between months. The temporal variability questions the effectiveness of the 50 kg/ha treatment to absorb and bioremediate oil contaminated wetland soils. In the 0 kg/ha controls, hydrocarbon concentrations ranged from 215 ppm to 2960 ppm. The significant increase in TPH concentration from August to September in the controls (402 ppm to 2960 ppm) helps to support the conclusion that the rain event in August resulted in the translocation of residual oil in the soil subsurface to be translocated to the soil surface.

For the 10-20 cm layer (Table 2.6 and Figure 2.6), all of the treatments remained below a mean TPH concentration of 300 ppm. The lowest TPH concentration was 40 ppm and the highest concentration was 241 ppm. There was no statistical difference between treatments for the 10-20 cm soil layer. The high spatial and temporal variability of oil within the wetland makes it difficult to prove that oil was being wicked by the ABG to the soil surface.

Table 2.6 Total Petroleum Hydrocarbon Means ($\bar{x}$) and Standard Deviations (s) for 10-20 cm Soil Layer in Oil Contaminated Wetland at Cravens, LA as a Result of Different Ammoniated Bagasse (ABG) Treatments.

ABG Treatment Applications	June		July		August		September	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
0 kg/ha	129^{Aa}	77	103^{Aa}	66	193^{Aa}	92	111^{Aa}	30
50 kg/ha	115^{Aa}	57	65^{Aa}	11	40^{Aa}	9	126^{Aa}	28
100 kg/ha	241^{Aa}	145	240^{Aa}	165	106^{Aa}	41	192^{Aa}	142
200 kg/ha	136^{Aa}	109	115^{Aa}	54	114^{Aa}	139	121^{Aa}	59

Means with the same lower case letters within rows are not significantly different. Means with the same upper case letters within columns are not significantly different.

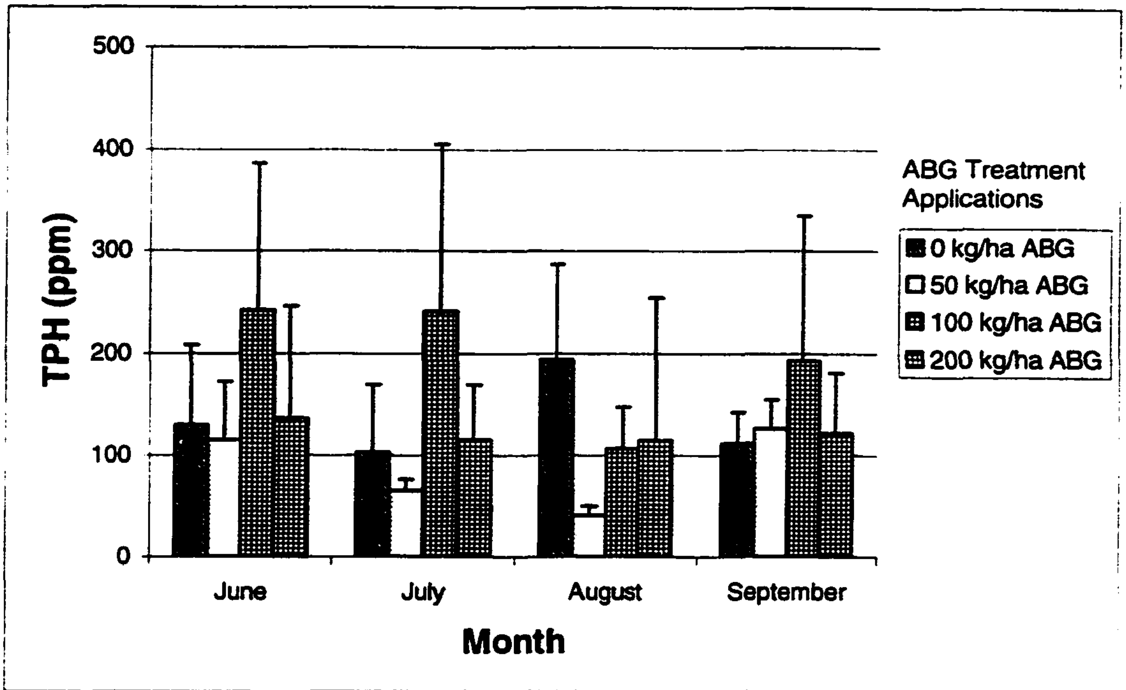

Figure 2.6 Total Petroleum Hydrocarbons in the 10-20 cm Soil Layer from the Oil Contaminated Wetland at Cravens, LA Resulting from Different Ammoniated Bagasse (ABG) Treatments (error bars are s.d.).

2.3.3 Statistical Analysis of Wetland Soil Hydrocarbon Data

Statistical analysis of the wetland soil hydrocarbon data was performed using the PROC GLM procedure. An α-value of 0.05 was used to determine significance of interactions of treatment, date, depth, and replications (Table 2.7). The effect of adding different amounts of bagasse (treatment) to reduce soil hydrocarbon concentrations was not found to be significant (p=0.1756). Date and depth were found to be significant with p-values of 0.0001 and 0.0001, respectively. Date by depth was also found to be significant (p=0.0001). Other interactions between treatment, date, depth, and replication were not found to be significant.

Time was seen to be significant in the removal of hydrocarbons from the soil. This is probably due to the increase in TPH from August to September in some of the treatments. Increases in TPH concentration in the 0 kg/ha, 100 kg/ha and 200 kg/ha treatments from August to September are probably due to the rain event that occurred at the end of August.

Hydrocarbons were greater in the 0-10 cm soil layer than in the 10-20 cm soil layer. The average hydrocarbon concentration of the 0-10 cm soil layer, pooling all treatments, was 595 ppm and the average concentration for the 10-20

Table 2.7 ANOVA Table of F and p Values for Wetland Soil Hydrocarbon Data Collected from the Oil Contaminated Wetland at Cravens, LA.

Source	F Value	Pr > F
Treatment	1.70	0.1756
Date	8.15	0.0001
Depth	29.94	0.0001
Replication(Treatment)	1.30	0.2290
Date*Treatment	1.50	0.1679
Date*Replication(Treatment)	0.97	0.5393
Date*Depth	7.99	0.0001
Treatment*Depth	1.13	0.3440
Date*Treatment*Depth	1.63	0.1268

cm soil layer was 175 ppm. This may be due to the absorbing ability of the bagasse to translocate oil from the 10-20 cm depth to the soil surface where microbial degradation can take place or the oil did not penetrate the soil deep enough to cause significant contamination in the 10-20 cm layer. The effect of the rain event in August may also be the cause of the highly significant differences in TPH between the two soil layers.

2.3.4 Electrical Conductivity and pH Analysis

All of the soil samples taken in June, July, August, and September were analyzed for electrical conductivity (Figures 2.7 and 2.8) and pH (Figures 2.9 and 2.10) using a saturated soil paste according to the methods of Page *et al.* (1982). The pH and EC data were pooled for all treatments for each month, as ABG does not affect these soil properties. For the 0-10 cm and 10-20 cm soil layers the electrical conductivity remained below 500 µS/m for the entire study period. There is a decrease from August to September in both soil layers. This is due to the rain event that occurred at the end of August. This rain caused the residual brine in the soil to be diluted, thereby reducing EC values. During this period the EC dropped below 300 µS/m. These results further illustrate that brine is no longer an obstacle to overcome to restore the site.

Foght and Westlake (1987) reported that the optimal pH range for microbial degradation of hydrocarbons in soil is 6 to 8. For the 0-10 cm and 10-20 cm soil layers, the pH during June remained below 6. During July and August, the pH for both soil layers was at or near 6, optimizing soil conditions needed for accelerated

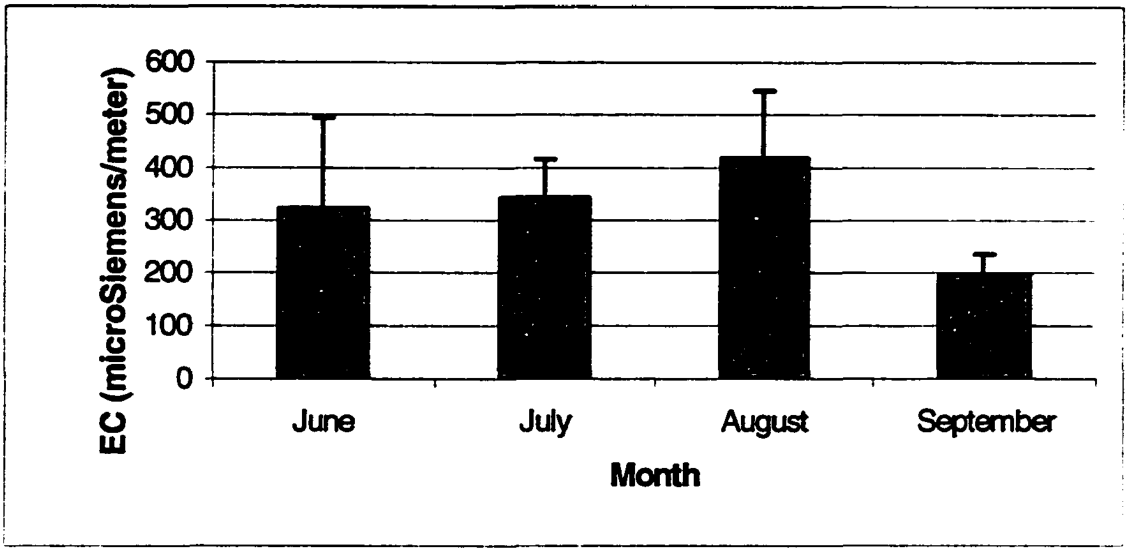

Figure 2.7 Electrical Conductivity in the 0-10 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are s.d.).

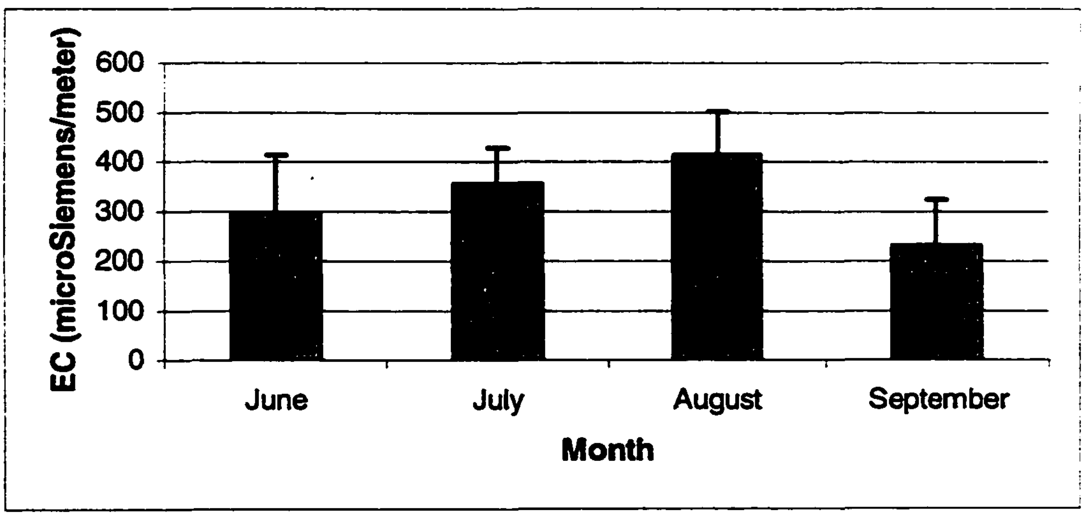

Figure 2.8 Electrical Conductivity in the 10-20 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are s.d.).

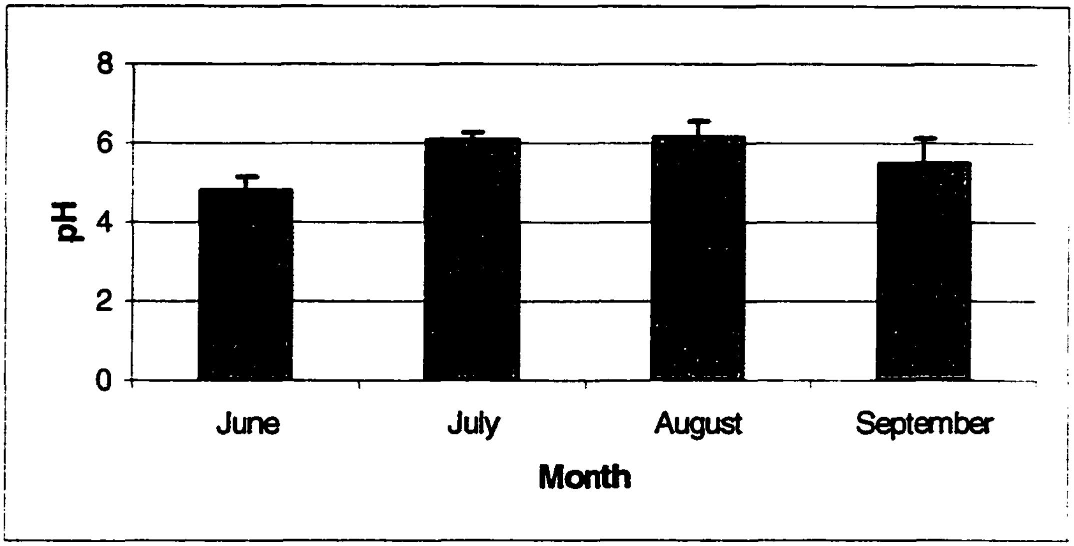

Figure 2.9 Soil pH of the 0-10 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are s.d.).

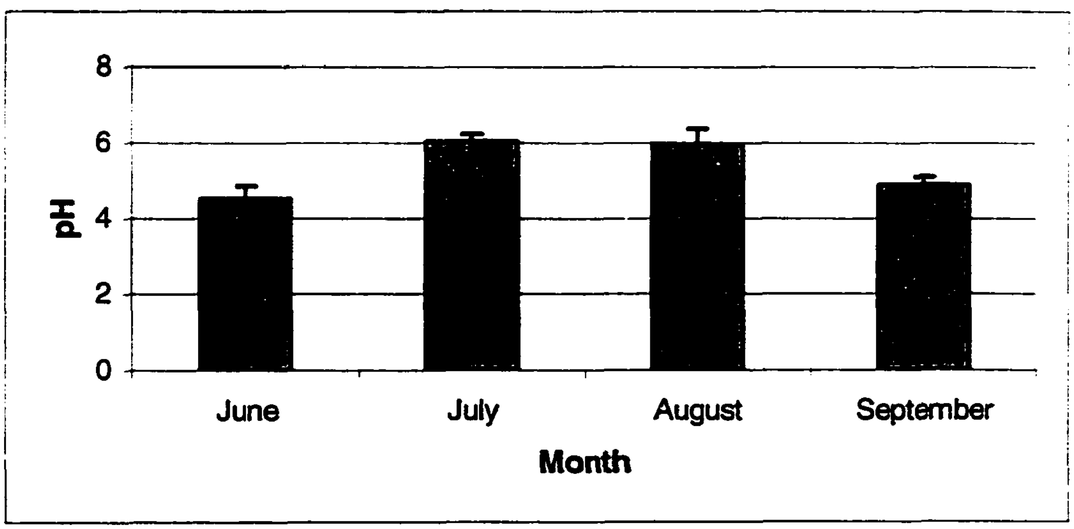

Figure 2.10 Soil pH of the 10-20 cm Soil Layer at the Oil Contaminated Wetland in Cravens, LA (error bars are s.d.).

degradation rates. From August to September there is a decrease in pH to below 6. This is probably due to the rain event that occurred in August.

The EC and pH data help to support the conclusion that the rain event in August may have caused oil to be moved to the soil surface. The water entering the wetland from the rain caused a decrease in the EC and pH of both the soil layers sampled. The drop in EC in the 10-20 cm layer proves that water from the rain event did indeed infiltrate the subsurface and had the potential to translocate oil to the surface. This would help to explain the increase in TPH in the 0-10 cm soil layer from August to September.

2.3.5 Cation Analysis

Results of the cation analysis indicated that there was no element present that would pose an environmental hazard or inhibit remediation of the wetland (Table 2.8). Iron concentrations were equal to or less than 1 ppm, with an average concentration of 0.152 ppm. These concentrations do not pose an environmental threat. Sodium concentrations ranged from 83 to 112 ppm, with an average concentration of 95 ppm. Even though chlorides were not determined, its concentration is assumed to be approximately equal to Na concentration. The average concentrations of Ca, K, and Mg are 2.5, 0.72, and 0.65 ppm, respectively. These concentrations would add only approximately 4 ppm to the chloride concentration.

Table 2.8 Mean Cation Concentrations for Wetland Soil Samples.[a]

Cation	0-10 cm Soil Depth	10-20 cm Soil Depth	Average	U.S. EPA Drinking Water Standards
Al	0.068	0.133	0.100	0.200
Ca	4.633	0.371	2.502	N/A
Cd	0.000	0.001	0.000	0.005
Cu	0.001	0.001	0.001	1.300
Fe	0.105	0.199	0.152	0.300
K	0.852	0.598	0.725	N/A
Mg	0.858	0.440	0.649	N/A
Mn	0.020	0.006	0.013	0.050
Na	94.964	95.921	95.442	50.000
Ni	0.002	0.001	0.002	0.100
Pb	0.006	0.004	0.005	0.015
Se	0.005	0.006	0.006	0.050
Zn	0.001	0.001	0.001	5.000

[a]Concentrations are expressed in parts per million (ppm).

2.4 Discussion and Conclusions

The wetland vegetation at the Cravens blowout site was completely destroyed as a result of the oil and brine contamination. The hydrology, which is the most important component of wetland restoration, was minimally affected (C-K Associates, Inc., 1999). There have been substantial reductions in brine contamination since the blowout occurred and it is no longer a deterrent to wetland remediation. As a result, restoration efforts should focus on the removal of residual oil from the soil and revegetation of the site by selected wetland species.

The use of ammoniated organic wastes, specifically bagasse, has been found to be a reliable application to enhance the remediation of ecologically sensitive environments that have been subjected to oil contamination (DeSilva, 1995 and Grace, 1997). It was expected that ABG would have a similar effect in the wetland impacted by the Cravens oilwell blowout if rapidly applied after the spill. Results of the study, though, do not statistically prove that ABG promoted biodegradation of the oil contaminated soil. The spatial variation of the oil within the wetland made it difficult to prove that successful remediation was achieved.

There was no statistical difference among ABG treatments during individual monthly sampling periods (Tables 2.5 and 2.6). September is the only month when there is any statistical difference between treatments. All of the treatments, except the 50 kg/ha treatment, had increases in TPH concentration in the 0-10 cm soil layer. This was most likely due to the rain event in August that resulted in residual oil in the subsurface to be translocated to the soil surface. This was supported by the data that shows EC's decreasing in the 0-10 cm layer from August to September as a result of dilution from the rain.

Even though this study did not statistically prove the effectiveness of ammoniated bagasse to bioremediate the wetland, it still may be a useful tool at this and other similar sites. The high spatial variability of oil within the Cravens wetland makes it difficult to determine the rates of ABG that would need to be applied in order to achieve successful remediation. Results of this study indicate that rates of ABG greater than 200 kg/ha would need to be applied in order to account for the spatial variation of the oil within the wetland. Larger treatment blocks would need to be established within the wetland using ABG rates greater than 200 kg/ha to determine the correct amount of ABG to be added to effectively remediate the wetland soils. Rates of ABG applied could be 0, 500, 750, 1000, 1500 kg/ha. Similar sampling procedures and laboratory techniques used in this study would be adequate for this type of investigation.

In oil spills such as these, the difficulty lies not only with removing the oil from the environment, but also restoring the area to its natural conditions. To restore the contaminated area, it must be decided if revegetation will occur by natural succession or if human intervention will be employed to accelerate the restoration process. This should be decided on a site-by-site basis as all spills and environmental conditions are not the same.

One important aspect that is often overlooked at restoration sites is monitoring and observation. In many cases, after restoration practices have been implemented the site is ignored and assumed to be a success. Most times this is not the case. Establishing vegetation at a site does not define success. The functional activity of the wetland within the surrounding landscape must also be assessed. This is especially true for oil contaminated wetlands which are often completely destroyed. At the Cravens blowout site, soil samples should be taken monthly after the bagasse has been added in order to determine its success at removing the residual oil from the soil. After oil concentrations have decreased to acceptable levels, a seed bank with selected species can be introduced into the wetland. Monitoring of vegetation should continue until successful establishment is certain.

Species to be introduced into the overstory of Cravens site may include black gum (*Nyssa sylvatica*), sweet gum (*Liquidambar styraciflua*), tupelo gum (*Nyssa aquatica*), sweet bay (*Laurus nobilis*), water oak (*Quercus nigra*), and bald cypress (*Taxodium distichum*). Understory vegetation may include American beautyberry (*Callicarpa americana*), muscadine (*Vitis rotundifolia*), and sassafras (*Sassafras albidum*). These species can be established by planting seeds, seedlings, or stem cuttings, plant-soil transfers, use of a donor seed bank, or natural succession. A key to successful revegetation is to reduce the amount of noxious, invasive plants that may colonize the site. Examples of some invasive species are Chinese tallow (*Sapium sebiferum*), hybrid cattail (*Typha glauca*), and purple loosestrife (*Lythrum salicaria*). With continued monitoring and observation, the odds for successful restoration of the oil contaminated wetland will increase.

As of this writing, the Forest Service has rejected the plan submitted to them to incorporate ABG into the remediation of the wetland. Instead, they used heavy equipment to excavate the top 0-15 cm of the oil contaminated wetland soil and will attempt to begin revegetation of the site in the next 3 to 6 months.

CHAPTER 3

EFFECTS OF FOLIAR AND SOIL APPLIED LOUISIANA SWEET CRUDE OIL ON TWO-YEAR OLD LOBLOLLY PINE (*PINUS TAEDA*) SEEDLINGS

3.1 Introduction

The effects of oil on vegetation have been studied since the early 1900's. The majority, if not all, of this research focused on the effects of oils on agricultural crops and coastal salt marsh communities. With increases in oil exploration and production in wetland and forest communities, the effects of accidental oil spills on vegetation within these environments must be better understood. Louisiana's Kisatchie National Forest, which is dominated by loblolly (*Pinus taeda*) and longleaf (*Pinus palustris*) pines, is subject to oil contamination during exploration and production operations. There is little knowledge of the phytotoxic effects of crude oil on pine trees and the long-term impacts on forest communities. Some general observations of the effects of oil on agricultural and salt marsh communities are applicable to pine trees.

Phytotoxic effects of oil on vegetation that have been observed include yellowing and death of leaves, reduction of seedlings, reduction or ceasing of growth rate, and death of the plant. These effects may vary according to the type and amount of oil involved, the degree of its weathering, the time of year, and the species and age of the plants concerned (Baker, 1970). In general, the smaller the hydrocarbon molecule, the more toxic the oil is to plants because smaller molecules can more easily enter the plant (van Overbeek and Blondeau, 1954).

Once oil has come in contact with a plant, it must be able to penetrate and move within the plant in order to cause injury. The oil molecule enters the plant more easily through the stomata or at the point of contact (Baker, 1970). Once inside the plant, the oil travels through intercellular spaces into the plant cells (Knight *et al.*, 1929). Within the cell, the oil damages the plasma membrane and cell sap leaks into the intercellular spaces (Baker, 1970). The leakage of cell sap causes the leaf to darken and lose turgor (Currier, 1951). This process, ultimately, upsets the physiology of the plant leading to stress or death.

Oils have also been found to alter transpiration, respiration, and photosynthetic rates of plants. Transpiration rates were found to be consistently reduced due to physical interference on or in the leaf tissue (Knight *et al.*, 1929 and Bartholomew, 1936). Respiration rates may increase or decrease depending on the tolerance of individual plant species. Respiration rates are reduced when oil interferes with gaseous exchange by blocking stomata and intercellular spaces (Baker, 1970). Oil has been found to consistently reduce the rate of photosynthesis, primarily by physical interference with gaseous exchange similar to respiration reduction.

The effects of crude oil on pine trees have never been investigated. There is no data available as to how much oil is lethal to pine trees or the long term effects of oil spills on pine forest communities. At oil well blowouts at Cravens and Union Hill, LA, loblolly and longleaf pines in the vicinity of the sites were severely stressed or died as a result of oil contamination. Symptoms of stress included chlorosis of needles, wilting of needles, and death of seedlings immediately adjacent to the oil wells. The objective of this study is to determine the levels of oil leading to stress and death of loblolly and longleaf pines affected by the oil well blowouts in a greenhouse study using nutrient concentration analysis.

3.2 Materials and Methods

The original greenhouse study investigated the effects and interactions of six different rates of foliar applied oil and brine to two-year old loblolly pine seedlings. The United States Forest Service donated approximately 1000 one-year old pine seedlings for this study. These seedlings were planted in March 1999 in tree pots (15 cm width / 41 cm height) filled with the A and B horizons of a Malbis silt loam (Fine-loamy, siliceous, subactive, thermic Plinthic Paleudult) in order to imitate forest soil conditions. The seedlings were watered with de-ionized water every other watering in order to reduce salt buildup in the soil. The seedlings grew for one year under monitored greenhouse conditions before any treatments were applied.

3.2.1 Foliar Oil Pre-study

From the oil well blowouts, it was assumed that the oil was one of the major causes of stress and death of the pine seedlings. The effects of weathered and unweathered crude oil on pine seedlings have never been investigated and there is no data available as to how much oil is lethal to pine seedlings. Therefore, an attempt to gather some preliminary data and information was performed in a pre-study involving foliar oil applications. The goal of this pre-study was to determine the LD_{50} (lethal dose needed to kill 50% of the population) of foliar applied oil for two-year old loblolly pine seedlings.

Ten fascicles from each of ten randomly selected seedlings were removed and the needle surface area of the needles was determined according to Johnson (1984). The total number of fascicles for each selected seedling was counted, and using a regression equation the total needle surface area of each seedling was calculated. The ten fascicles from each selected seedling were immersed in oil in order to determine the amount of oil necessary to cover 100% of the needle surface area. From this data and using a regression equation, the amount of oil necessary to cover 100% of the total needle surface area for each selected seedling was determined. Based on this data, it was determined how much oil to apply foliarly to achieve various estimated percentages of needle surface area covered with oil.

In early April 2000, oil was foliarly applied to randomly selected seedlings from the greenhouse population at rates of 1.3, 1.9, 2.5, 3.1, 3.8, 4.4, 5.0, 5.7, and 6.3 mL/seedling in order to achieve needle surface area coverages of approximately 20, 30, 40, 50, 60, 70, 80, 90, and 100%. There were five replications for each treatment. The height of each seedling was measured before oil application. Surprisingly, the seedlings showed little signs of stress six weeks after treatment. The only effect observed was on the seedlings sprayed to cover 100% of needle surface area. The new shoot growth of the 100% treatments showed signs of stress (necrosis and wilting), but no other parts of the seedlings were visually stressed. A second treatment using the same rates was applied after the six weeks. The same visual effects were noted, but there was no consistent effect. Therefore, a LD_{50} for foliar applied oil on two-year old loblolly pine seedlings could not be determined. These seedlings were continually monitored and harvested in October 2000, separating into needles, stems, and boles. The height of each seedling was measured again before harvesting. A visual health rating was assigned to each seedling before harvesting. A numbering system from one to five was used, with one being a healthy seedling showing no signs of stress and five being a seedling with all needles brown and assumed to be dead.

Needles, boles, and stems of each seedling were analyzed for Al (aluminum), As (arsenic), B (boron), Ca (calcium), Cd (cadmium), Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), K (potassium), Mg (magnesium), Mn (manganese), Mo (molybdenum), N (nitrogen), Na (sodium), Ni (nickel), P (phosphorus), Pb (lead), S (sulfur), Se (selenium), Si (silicon), and Zn (zinc) using a modified nitric/H_2O_2 digestion technique (Bell, 2000) and a Perkin-Elmer ICAP analyzer. Modifications to the digestion procedure employed by Huang and Schulte (1985) included diluting the final sample to 25 mL instead of 50 mL, heat till 2 or 3 mL of solution remains, and rinsing the sides of the digestion tubes with de-ionized water after frothing has stopped. Statistical analysis of the needle tissue data was performed using the SAS GLM procedure.

3.2.2 Foliar Oil Study

It was decided to repeat the pre-study experiment by applying oil at rates of 0, 1.7, 3.1, 4.7, and 6.3 mL/seedling to achieve needle surface area coverages of 0, 25, 50, 75, and 100%, respectively. There were 15 replications of each treatment. Seedlings for this study were randomly selected from the greenhouse population and assigned a treatment. Seventy-five total seedlings were used in this study. Oil was foliar applied to the seedlings on May 15, 2000 with a second application on May 22, 2000. The height and diameter of each seedling were measured before any oil was applied.

The seedlings were monitored and watered with de-ionized water every other watering to reduce salt buildup in the soil. The seedlings were harvested in October 2000. The height and diameter were once again measured before the seedlings were separated into needles, stems, and boles. The same visual health rating system used in the pre-study was also used in this study. Nutrient concentration analysis for each part of each seedling was performed. The seedlings were analyzed for Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, S, Se, Si, and Zn using the same modified block digestion technique (Bell, 2000) and statistical analysis as in the pre-study.

3.2.3 Soil Oil Application Study

A second oil study was performed to evaluate the effects of oil applied directly to the soil on two-year old pine seedlings. Ammoniated bagasse (ABG) was also incorporated in this study in order to determine its efficiency at reducing total petroleum hydrocarbon (TPH) concentrations of oil contaminated forest soils. A 4x4 factorial design with four rates of oil and four rates of ABG with five replications of each treatment was employed. The seedlings used in this study were randomly selected from the greenhouse population and assigned a treatment. Eighty total seedlings were used in this study. The rates of oil added to the soil were 0, 431, 862, and 1724 L/ha. These rates of oil are equivalent to

0, 100, 200, and 400 mL/seedling. The oil was applied directly to the soil surface on May 8, 2000. Height and diameter measurements for each seedling were taken before the oil was applied. The ABG was added to the soil 14 days after the oil treatments at rates of 0, 431, 862, and 1724 kg/ha and mixed into the upper 2 cm of the soil. These rates of ABG are equivalent to 0, 100, 200, and 400 g/seedling. The ABG was added 14 days after the oil applications in order to imitate response times in typical oil spill situations.

The seedlings were monitored and watered with de-ionized water every other watering to reduce salt buildup in the soil. The seedlings were harvested in October 2000. The same visual health rating system used in the other studies was also used in this study. The height and diameter were once again measured before the seedlings were separated into needles, stems, and boles. Nutrient concentration analysis for each part of each seedling was performed. The seedlings were analyzed for Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, S, Se, Si, and Zn using the modified block digestion technique (Bell, 2000) and a Perkin-Elmer ICAP analyzer. Soil samples for each treatment were taken approximately six months after ABG application and analyzed for TPH concentration employing the same methods used to analyze the TPH of the wetland soils (Chapter 2, Section 2.2.2). Statistical analysis of the needle tissue data and soil data was performed using the SAS GLM procedure.

3.3 Results

Many studies investigating the effects of single or multiple stresses on plants often focus on the root-shoot response to the stress(es). Responses in these parts of the plant help describe the whole-plant response. In the greenhouse studies, nutrient analysis of needles, stems, and boles took place to investigate the seedling response to oil stress. Only the needle nutrient concentration results are presented and discussed here, as there were no unusual results from the stem and bole data that require discussion. Tables A3 through A6 present the bole and stem nutrient concentrations for the oil studies.

3.3.1 Foliar Oil Pre-study

The pre-study seedlings treated with foliar applications of oil in April showed no signs of stress six weeks after treatment. The only negative effect was on seedlings saturated with oil to cover 100% of the needle surface area. New shoot growth on these seedlings became brown and wilted four to six weeks after application. It was only eight to ten weeks after application that other treatments began to show signs of stress. Seedlings treated to cover 70%, 80%, and 90% of the needle surface area showed signs of severe stress, including needle chlorosis, necrotic lesions, wilting needles, and premature needle loss (Table 3.1). All of these seedlings, and the 100% treated seedlings, eventually died before the October harvest date. Seedlings treated to cover 60% of the needle surface area showed similar signs of stress, but not quite as severe. Three of the five 60% replications died before the October harvest. Seedlings treated to cover 20%, 30%, 40%, and 50% of the needle surface area showed little or no signs of stress. Signs of stress that were observed were needle

Table 3.1 Mean Health Ratings and Change in Height of Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar Applied Oil (Pre-study).

Needle Surface Area Covered with Oil	Mean Health Rating*	Mean Diameter Change (mm)
20%	2.0[d]	2.2[b]
30%	2.4[d]	5.2[a]
40%	2.2[d]	5.4[a]
50%	2.8[bcd]	6.2[a]
60%	3.8[abcd]	2.8[a]
70%	4.6[abc]	8.6[a]
80%	4.8[ab]	5.4[a]
90%	4.8[ab]	6.2[a]
100%	5.0[a]	2.2[b]

*Ratings: 1 (healthy, no visual sings of stress); 2 (slight visual signs of stress, few chlorotic and wilting needles); 3 (moderate signs of stress, chlorotic needles, wilting needles, some premature needle loss); 4 (severe signs of stress, chlorotic and necrotic needles, extreme wilting, premature needle loss); 5 (seedling is dead, all needles brown).
Means with the same letter within columns are not significantly different at α=0.05.

chlorosis and wilting. None of these seedlings died. Change in height measured before and after oil applications was not significant (p=0.1640).

Nutrient analysis of the seedlings showed some interesting trends (Table 3.2, Figures 3.1-3.4). In the seedlings that eventually died (70-100% treatments), concentrations of Ca, Cd, Fe, P, and Pb were increased when compared to the

Table 3.2 Mean Needle Nutrient Analysis of Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar Applied Oil (Pre-study).[a]

Nutrient	20%	30%	40%	50%	60%	70%	80%	90%	100%
Al[t]	374	362	296	360	331	402	343	304	334
B	59	31	27	35	27	31	34	27	18
Ca	2034	1602	1888	1552	1744	2321	2017	1745	2624
Cd	0.14	0.12	0.13	0.11	0.09	0.14	0.14	0.15	0.15
Co	0.25	0.25	0.18	0.18	0.18	0.26	0.20	0.16	0.14
Cr	1.03	0.91	2.69	1.19	1.63	2.14	1.72	1.55	1.53
Cu[t]	3.84	3.62	3.04	3.36	3.37	3.80	3.95	3.64	3.44
Fe	61	48	66	50	48	90	70	62	98
K	3713	3665	3012	3080	2864	3957	3599	3468	2586
Mg[t]	824	810	762	818	761	983	815	749	691
Mn	363	328	307	255	254	323	323	269	256
N	13804	14090	13106	13954	14072	14262	14928	16058	13328
Na	5957	5643	3414	6502	4347	4644	4586	3205	2951
Ni[t]	2.04	1.94	3.91	1.73	2.39	1.85	1.65	1.39	1.35
P	1181	1185	902	1044	965	1194	1287	1411	1292
Pb	16	11	17	15	19	24	18	23	42
S	5656	5531	2311	5656	4875	5932	5929	4322	831
Se	0.13	0.25	0.38	0.40	0.15	0.32	0.40	0.36	0.77
Si	119	140	120	122	106	137	123	73	144
Zn	89	73	54	50	68	91	67	76	81

[a]Mean values are parts per million (ppm).

[t]Treatment effects are not significant a α=0.05.

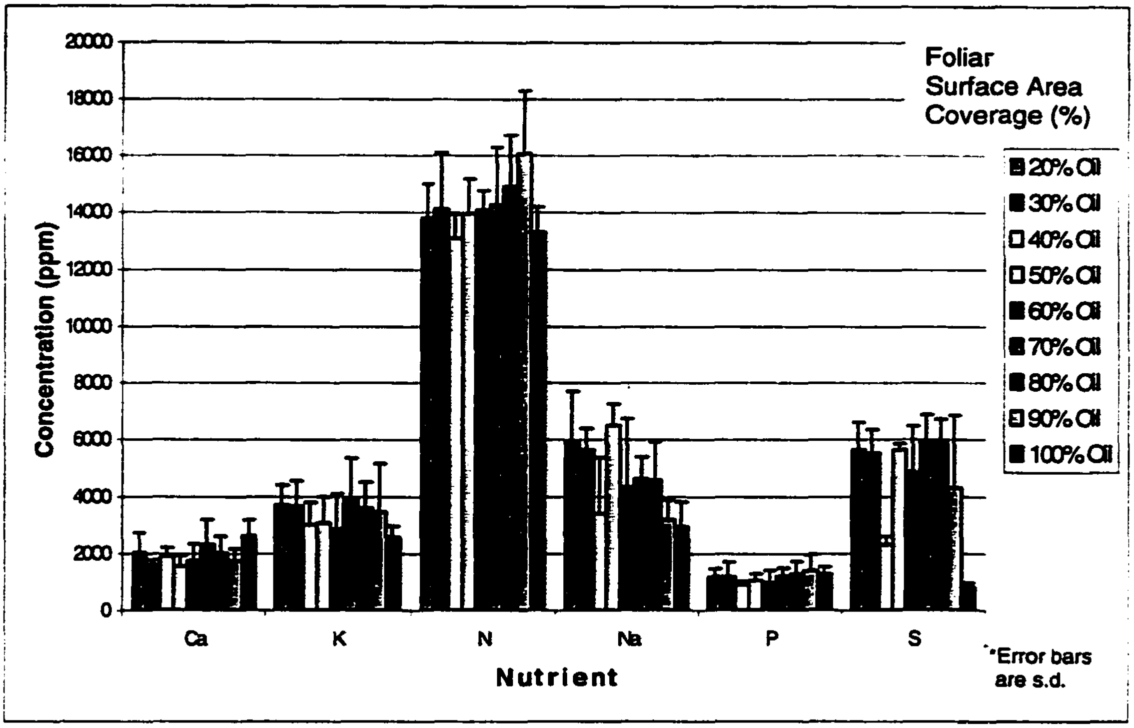

Figure 3.1 Ca, K, N, Na, P, and S Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil (Pre-study).

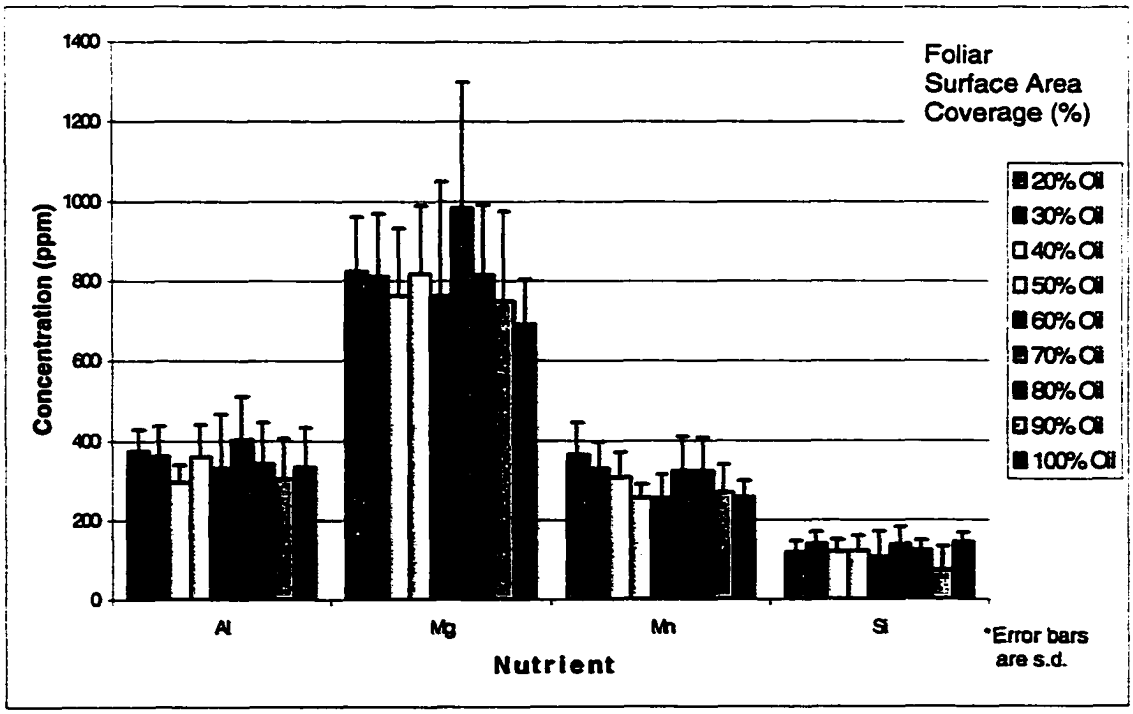

Figure 3.2 Al, Mg, Mn, and Si Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil (Pre-study).

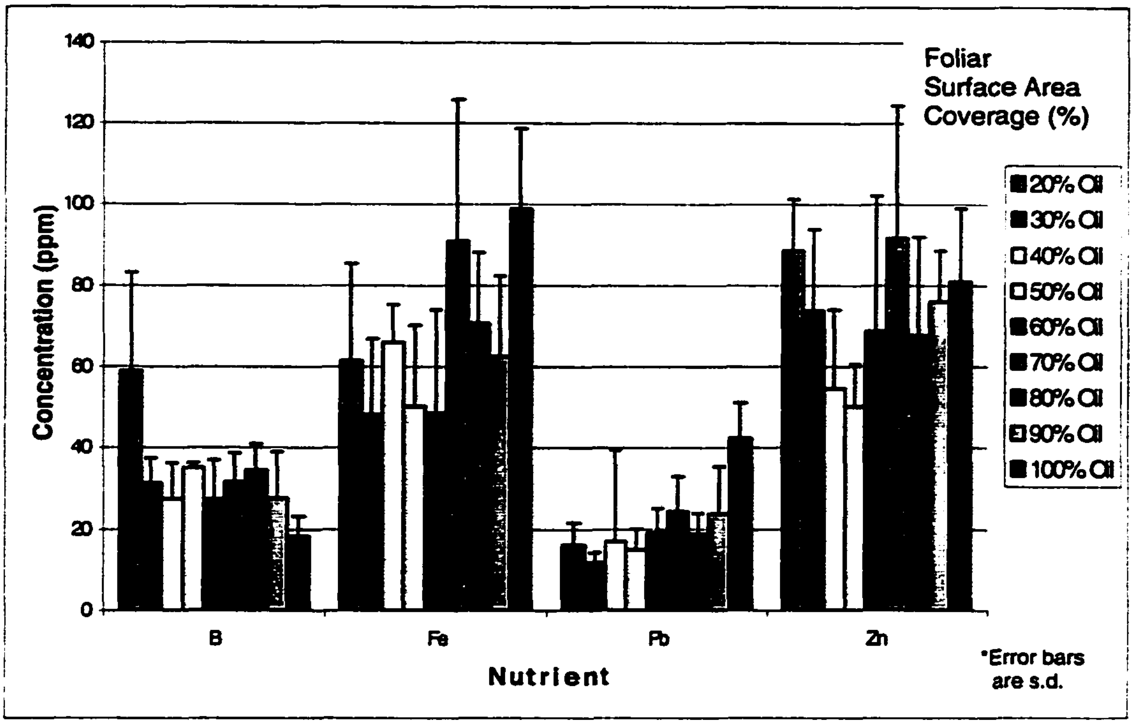

Figure 3.3 B, Fe, Pb, and Zn Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil (Pre-study).

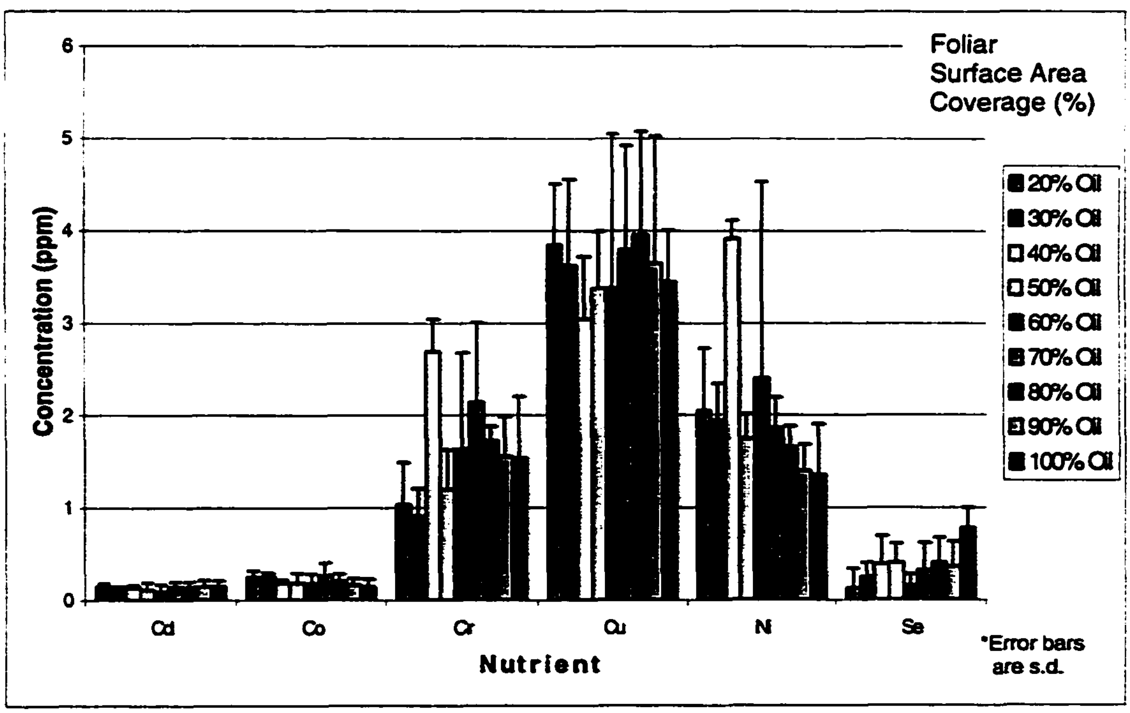

Figure 3.4 Cd, Co, Cr, Cu, Ni, and Se Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil (Pre-study).

decreased from the 80% to 100% treatment. Nickel decreased from the 60% treatment to the 100% treatment. Nitrogen concentrations showed an increasing trend up to 90%. From 90% to 100% there is nearly a 3000 ppm decrease (16058 ppm to 13328 ppm) in N. Concentrations of As and Mo fell below the detection limits for all treatments, therefore were not included in Table 3.2.

After evaluating the role of each nutrient in seedlings, it was concluded that the oil is somehow interfering with the photosynthetic process. Copper, Mg, and Mn are all critical constituents of the photosynthetic pathway. Copper is important in the electron transfer process, Mg is a constituent of chlorophyll, and Mn is essential for chlorophyll synthesis. The concentrations of each of these nutrients exhibited a decreasing trend from the 70% to 100% treatments, but the decrease in Mn was the only nutrient statistically significant. This decline may indicate that the photosynthetic process may be inhibited at some point after needle surface area coverage of oil exceeds 60%. Dallyn (1953) states that chlorophyll destruction is one of the most obvious symptoms of oil injury. As the photosynthetic rate decreases, seedlings will begin to show signs of stress. Visual indications of stress of the seedlings included needle chlorosis and needle wilting and eventually death.

Based on previous studies (Baker, 1970), it is assumed that the oil contamination is playing the primary role in upsetting the photosynthetic process. The oil may be physically interfering with gaseous exchange by blocking stomata, which would cause a decrease in photosynthetic rates. If the oil is entering the needles through the stomata, it may be damaging cells. After the oil enters the needles through the stomata and moves into cells, it destroys cell membranes, causing cell sap to leak into intercellular spaces. Once these cells are destroyed, photosynthesis may not occur at rates needed to sustain the seedling. A decreasing photosynthetic rate may explain the decrease in the concentrations of Cu, Mg, and Mn in the seedlings that exhibited the most severe signs of stress.

The stress caused by the oil also explains the increased concentrations of Pb in the seedlings that died. Plants impede the translocation of Pb from absorbing roots to other parts of the plant by biochemical and physical processes involving Pb binding, inactivation, and/or precipitation (Koeppe, 1981). As the seedlings become more stressed, their ability to inhibit the uptake and translocation of Pb decreases, allowing Pb to enter the plant. Kramer and Kozlowski (1979) report that lead pollution decreases the photosynthetic process directly or indirectly, by causing loss of photosynthetic tissues (i.e. necrosis or chlorosis) and by affecting stomatal aperture.

3.3.2 Foliar Oil Study

A second foliar oil study was conducted using needle surface area coverages of 0, 25, 50, 75, and 100%. These treatments were applied twice in mid-May 2000. Table 3.3 summarizes health ratings and height and diameter changes for each treatment. Whereas the seedlings in the pre-study took eight to ten weeks to show visual signs of stress, the seedlings in this study showed signs of stress within two weeks of application. The 100% oil treated seedlings began to show needle wilting and chlorosis within two weeks of application and

Table 3.3 Mean Health Ratings, Height Change, and Diameter Change of Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar Applied Oil.

Needle Surface Area Covered with Oil	Mean Health Rating*	Mean Height Change (cm)	Mean Diameter Change (mm)
0%	1.1[d]	1.3[a]	4.39[a]
25%	1.6[d]	2.3[a]	3.85[a]
50%	2.9[c]	3.2[a]	3.02[a]
75%	3.9[b]	2.3[a]	4.19[a]
100%	5.0[a]	1.4[a]	3.23[a]

*Ratings: 1 (healthy, no visual sings of stress); 2 (slight visual signs of stress, few chlorotic and wilting needles); 3 (moderate signs of stress, chlorotic needles, wilting needles, some premature needle loss); 4 (severe signs of stress, chlorotic and necrotic needles, extreme wilting, premature needle loss); 5 (seedling is dead, all needles brown).
Means with the same letter within columns are not significantly different at α=0.05.

new shoot growth became necrotic and wilted. All of the 100% seedlings died approximately eight to ten weeks after treatment. The 75% treated seedlings showed signs of stress approximately two weeks after the 100% seedlings began to stress. Signs of stress included chlorotic and wilted needles. With time, these seedlings began to exhibit more severe signs of stress, including premature needle loss. Most of these seedlings probably would have died if harvested later than October.

The 50% treated seedlings showed some signs of stress, but less severe than that of the 75% and 100% treatments. Signs of stress included needle chlorosis and wilting, with some premature needle loss. None of these seedlings

Table 3.4 Mean Needle Nutrient Analysis of Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar Applied Oil (% foliar coverage).*

Nutrient	0%	25%	50%	75%	100%
Al	288	355	364	386	353
B	28.3	30.7	27.6	30.4	23.3
Ca[t]	1823	1930	1970	1987	2032
Cd[t]	0.21	0.19	0.20	0.27	0.27
Co	0.21	0.31	0.29	0.28	0.41
Cr[t]	0.76	1.73	1.11	1.83	2.27
Cu	2.42	2.61	2.56	5.79	2.14
Fe	41	55	60	105	68
K	2017	3152	5641	2413	1812
Mg[t]	744	891	900	845	773
Mn[t]	377	413	406	432	416
N	11049	10635	9893	10119	8583
Na	5731	4817	4615	5346	4182
Ni[t]	1.37	1.94	1.47	1.68	2.24
P[t]	774	861	855	898	745
Pb	8.47	8.55	7.69	8.64	12.30
S[t]	613	682	644	697	637
Se	0.35	0.92	0.75	1.06	0.42
Si	420	132	138	133	149
Zn	49.6	47.9	45.0	49.8	82.2

*Mean values are parts per million (ppm).
[t]Treatment effects are not significant a α=0.05.

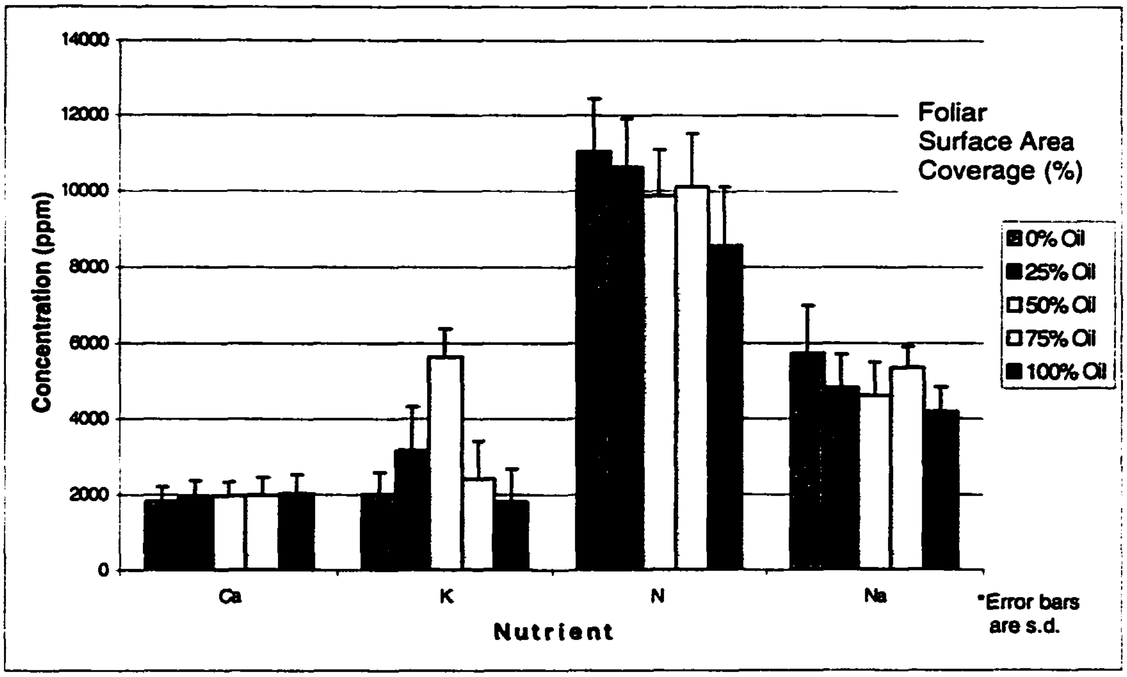

Figure 3.5 Ca, K, N, and Na Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil.

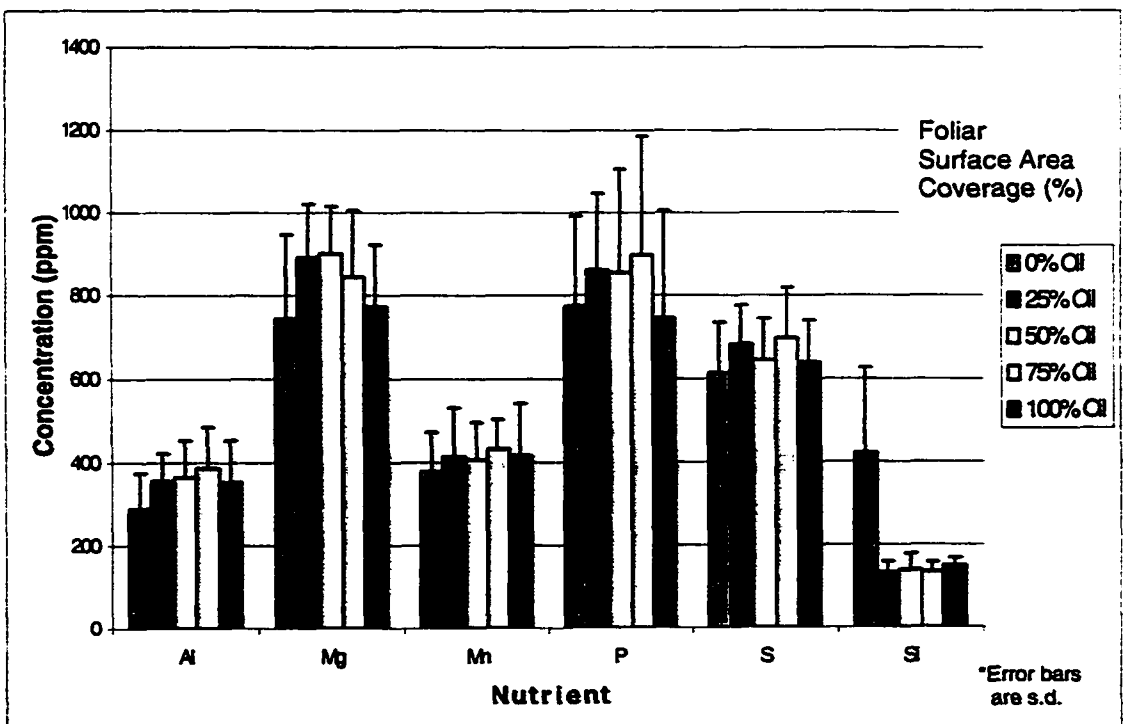

Figure 3.6 Al, Mg, Mn, P, S, and Si Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil.

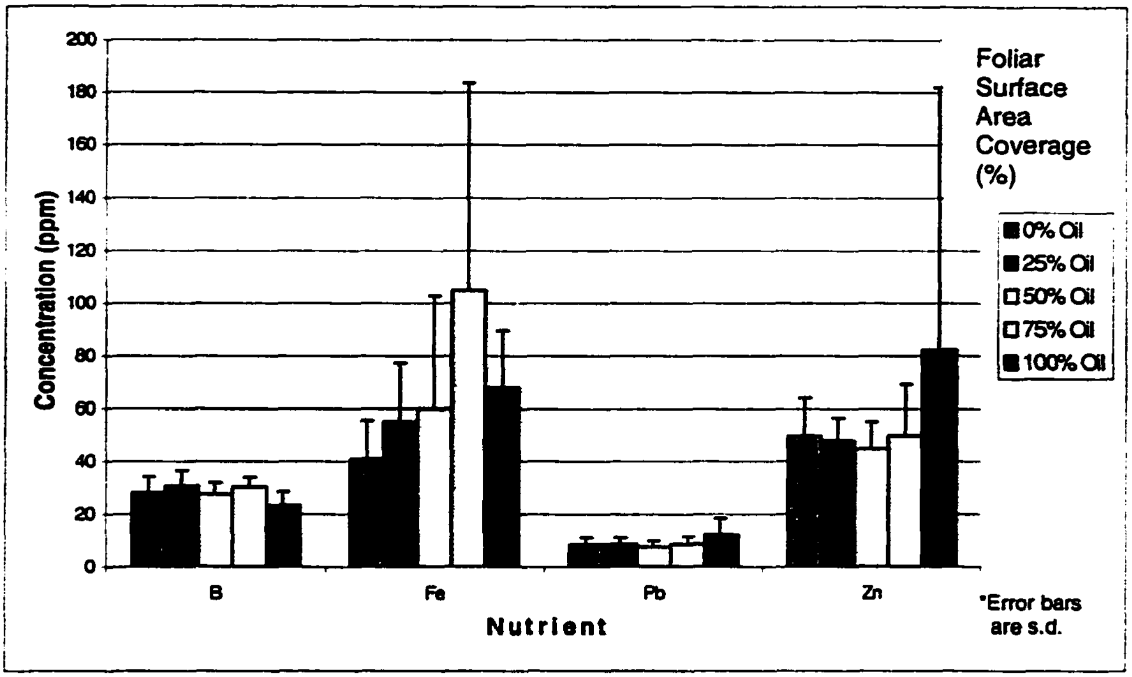

Figure 3.7 B, Fe, Pb, and Zn Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil.

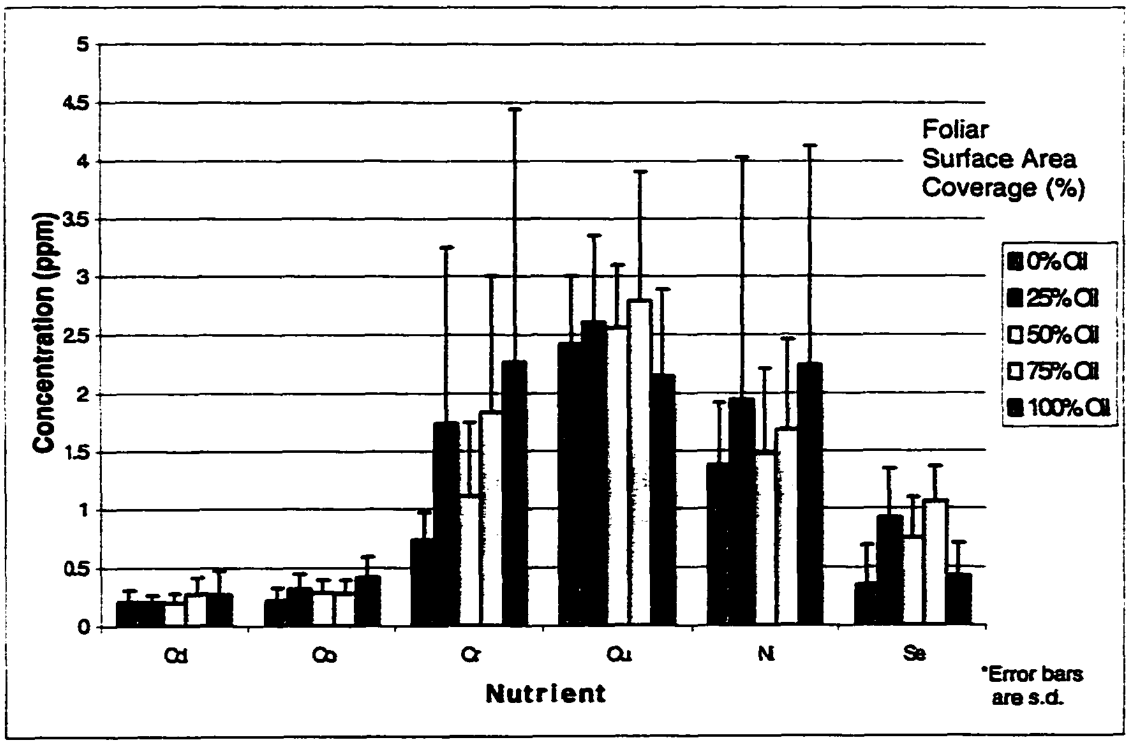

Figure 3.8 Cd, Co, Cr, Cu, Ni, and Se Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Oil.

died. The 25% treated seedlings showed little or no signs of stress. Some seedlings had needle chlorosis and wilting, but none of the seedlings died. No signs of stress were seen in the controls. For all treatments, changes in height were significant ($p=0.0384$), while changes in diameter were not significant ($p=0.1758$).

Nutrient analysis of the seedlings showed trends similar to those seedlings in the pre-study (Table 3.4, Figures 3.5-3.8). Boron, Cu, Fe, Mn, Na, P, and S concentrations decreased from the 75% to 100% treated seedlings. Potassium and Mg concentrations decreased from the 50% to 100% treated seedlings. Calcium concentrations increased with treatment. The heavy metals, Co, Cr, Ni, Pb, and Zn, concentrations increased from the 75% to 100% treated seedlings. Aluminum concentrations increased when the oil was added (0% to 25%) and then remained relatively level for the remainder of the treatments. Nitrogen concentrations tended to decrease with increasing foliar surface area coverage. Additions of oil caused a sharp decline in Si concentration (420 ppm to 132 ppm). Arsenic and Mo concentrations fell below detection limits and are not included in Table 3.4.

An evaluation of the role of each nutrient in the plant indicates that the oil may be interfering with the photosynthetic pathway. As in the foliar oil pre-study, there are declines in Cu, Mg, and Mn, all important constituents of the pathway. In addition to decreases in these nutrients, there is also a decrease in Fe and N concentrations. Iron plays a major role in chloroplast synthesis. Nitrogen is required for growth and a large percentage of N in leaves occurs as enzymes in the chloroplasts and mitochondria (Kramer and Kozlowski, 1979). It may be concluded that the oil is physically interfering with gaseous exchange or destroying cells within the needles, which causes a decrease in photosynthetic rate by decreasing concentrations of Cu, Fe, Mg, Mn, and N. Decreasing concentrations of these essential nutrients interferes with chloroplast synthesis, chlorophyll synthesis, and/or electron transport. Chlorophyll destruction is one of the first symptoms seen in oil contaminated vegetation. Declines in these nutrients also causes needle chlorosis and wilting (Baker, 1970), all symptoms seen in the stressed seedlings.

Statistical analysis of the effects of treatment on the concentrations of the photosynthetic nutrients makes it difficult to positively prove that the oil is interfering with the pathway. Treatment effects were not significant for Mg or Mn, but were significant for Fe and N. Variation within treatments made it difficult to analyze the trends for Mg and Mn. As a result, other physiological processes, such as respiration or transpiration, may have also been affected by the oil contamination. Increases in respiration may have exceeded photosynthesis resulting in stressing of the seedlings.

The sharp decline in Si (420 ppm to 132 ppm) when oil is added may have contributed to the wilting of the pine needles. Silicon provides structural support and helps keep needles rigid. Deficiencies of Si may cause wilting of needles (Kramer and Kozlowski, 1979), a symptom seen in the seedlings.

The increase of the heavy metals, Co, Cr, Ni, Pb, and Zn, can be attributed to the seedlings becoming so severely stressed that they can no longer inhibit the uptake of these metals. This trend was also observed for the foliar oil pre-study with increasing Pb concentrations. This may account for the increase of these metals from the 75% to 100% treated seedlings.

3.3.3 Soil Oil Application Study

3.3.3.1 Nutrient Analysis

Adding crude oil directly to the soil resulted in rapid negative reactions from the seedlings. The seedlings treated with the higher rates of soil applied oil (862 L/ha and 1724 L/ha) showed signs of severe stress and as a result most the seedlings died (Table 3.5). The 1724 L/ha oil treated seedlings showed severe signs of stress within one week after application and all of the seedlings were dead three weeks after application. Signs of stress included needle wilting, chlorosis, necrosis, and premature needle loss. The 862 L/ha oil treated seedlings showed similar signs of stress, but took longer to die. Only four of the twenty 862 L/ha oil treated seedlings did not die by the October harvest date. The 0 L/ha and 431 L/ha oil treated seedlings showed little or no signs of stress. Wilting of some needles was the only visual stress seen as a result of the oil application. Changes in height and diameter were statistically significant, with both measurements having p-values of <0.0001.

As would be expected, treatments in which seedlings died were significantly different compared to treatments that did not result in death. The control had the greatest increase in height and diameter, followed by the 431 L/ha oil treatment. Changes in height and diameter of the 862 L/ha and 1724 L/ha oil treatments were significantly different compared to the control and 431

Table 3.5 Mean Health Ratings, Height Change, and Diameter Change of Greenhouse Grown Two-year Old Loblolly Pine in Response to Soil Applied Oil.

Soil Applied Oil Treatment	Mean Health Rating*	Mean Height Change (cm)	Mean Diameter Change (mm)
0 L/ha	1.2^{c}	3.2^{a}	1.48^{a}
431 L/ha	1.2^{c}	1.8^{b}	1.35^{a}
862 L/ha	4.3^{b}	1.1^{bc}	0.77^{b}
1724 L/ha	5.0^{a}	0.0^{c}	0.41^{b}

*Ratings: 1 (healthy, no visual sings of stress); 2 (slight visual signs of stress, few chlorotic and wilting needles); 3 (moderate signs of stress, chlorotic needles, wilting needles, some premature needle loss); 4 (severe signs of stress, chlorotic and necrotic needles, extreme wilting, premature needle loss); 5 (seedling is dead, all needles brown).
Means with the same letter within columns are not significantly different at α=0.05.

L/ha oil treatment. It can be concluded that oil applied soil rates exceeding 431 L/ha will cause the seedlings to stress and reduce the growth rate.

It was obvious from the results of this study that oil applied directly to the soil had a more acute effect than foliar applied oil. Death of the seedlings was probably a direct cause of root interference caused by the oil. Roots perform three main functions: anchor the tree in the soil, absorb water and nutrients, and food storage. Oil spills can directly and indirectly affect all of these functions.

The tree root system consists of long-lived woody roots, which support a mass of short-lived, non-woody absorbing roots. The smaller, absorbing roots are more likely to be affected by oil spills because the majority of these roots occur in the top 10 cm of the soil. This soil depth is also where spilled oil will concentrate in the soil. The oil can reduce respiration rates and impede absorption of water and nutrients by roots. Respiration may be reduced in a manner similar to the way that oil affects respiration rate on leaves or needles, by coating the roots and inhibiting gaseous exchange. By impeding the uptake of water and nutrients by roots, oil will cause seedlings to react as they would in drought conditions. This would include decreasing growth rate, decreasing seed germination, inhibiting the movement of water and nutrients to the upper areas of the tree, and increasing the susceptibility to disease and insects. The seedlings that were stressed showed a decline in growth rate and also reductions in essential nutrients.

Nutrient analysis of the soil applied oil seedlings showed trends similar to the other oil studies (Table 3.6, Figures 3.9-3.12). Aluminum, Ca, Mg, Mn, Se and Si concentrations decreased from the 862 L/ha to 1724 L/ha oil treatments. Boron and Na decreased with these treatments. The heavy metals, Cd, Cr, Ni, Pb, and Zn, concentrations increased from the 862 L/ha to 1724 L/ha oil treatments. Cadmium, Fe, P, and S increased with increasing oil treatment. Nitrogen concentrations increased with increasing oil concentration. Arsenic and Mo fell below detection limits and are not included in Table 3.6.

Photosynthesis may have been inhibited by a reduction in Mg and Mn. The increase in the heavy metals is a result of the seedlings becoming so stressed that they could no longer inhibit uptake of these elements. These trends may be indirect results of the oil contaminating the absorbing roots

Table 3.6 Mean Needle Nutrient Analysis of Greenhouse Grown Two-year Old Loblolly Pine in Response to Soil Applied Oil.*

Nutrient	0 L/ha Oil	431 L/ha Oil	862 L/ha Oil	1724 L/ha Oil
Al	233	259	289	257
B[t]	21.4	21.4	19.5	17.9
Ca[t]	1734	1898	2002	1904
Cd	0.122	0.096	0.109	0.138
Co	0.104	0.189	0.147	0.159
Cr	0.94	3.69	1.33	4.91
Cu	2.24	2.55	2.47	2.75
Fe	25.5	52.3	50.3	89.9
K	1989	2608	2322	2657
Mg	696	799	813	699
Mn	207	213	313	286
N	11033	12303	12142	12673
Na	4285	3803	2670	1940
Ni	1.49	4.36	2.13	6.51
P	709	682	705	755
Pb	3.8	12.9	12.3	23.1
S	581	643	649	664
Se	0.758	0.766	0.839	0.356
Si	133	166	182	109
Zn	46.0	58.7	36.7	43.4

*Mean values are parts per million (ppm).
[t]Treatment effects are not significant a α=0.05.

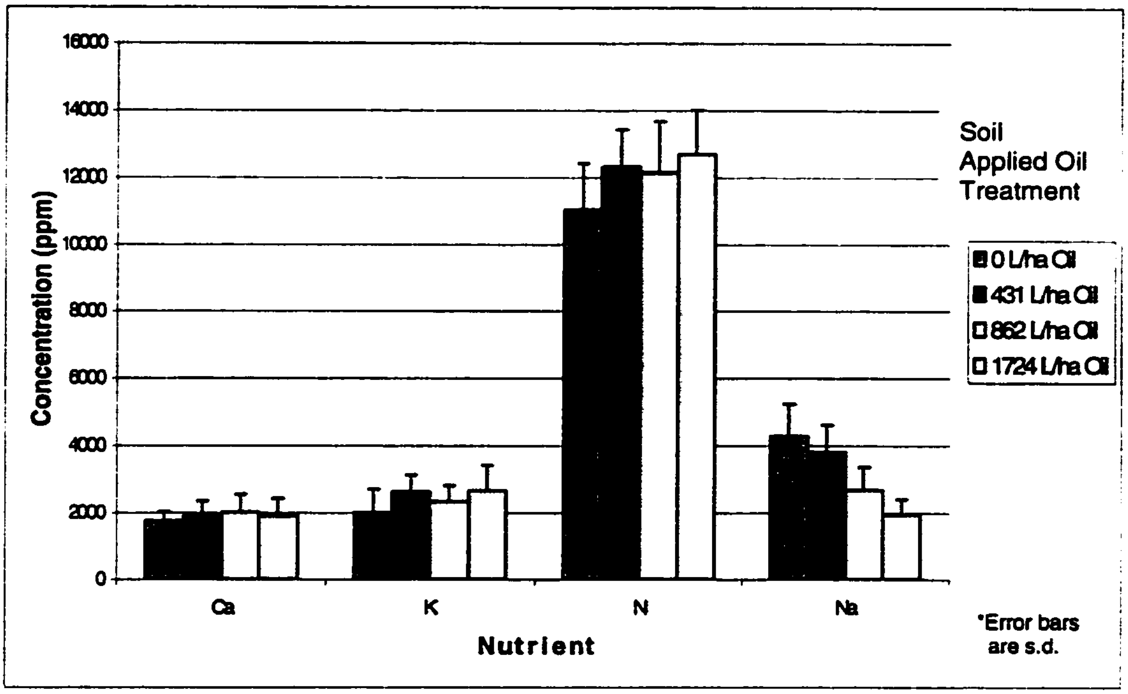

Figure 3.9 Ca, K, N, and Na Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Oil.

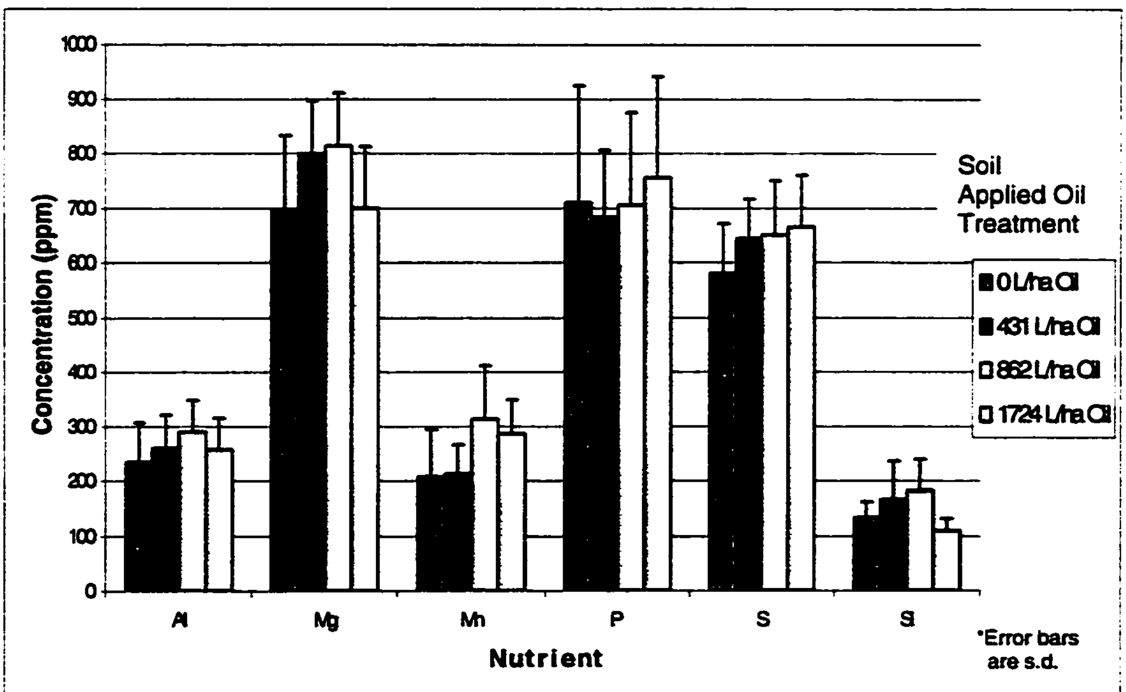

Figure 3.10 Al, Mg, Mn, P, S, and Si Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Oil.

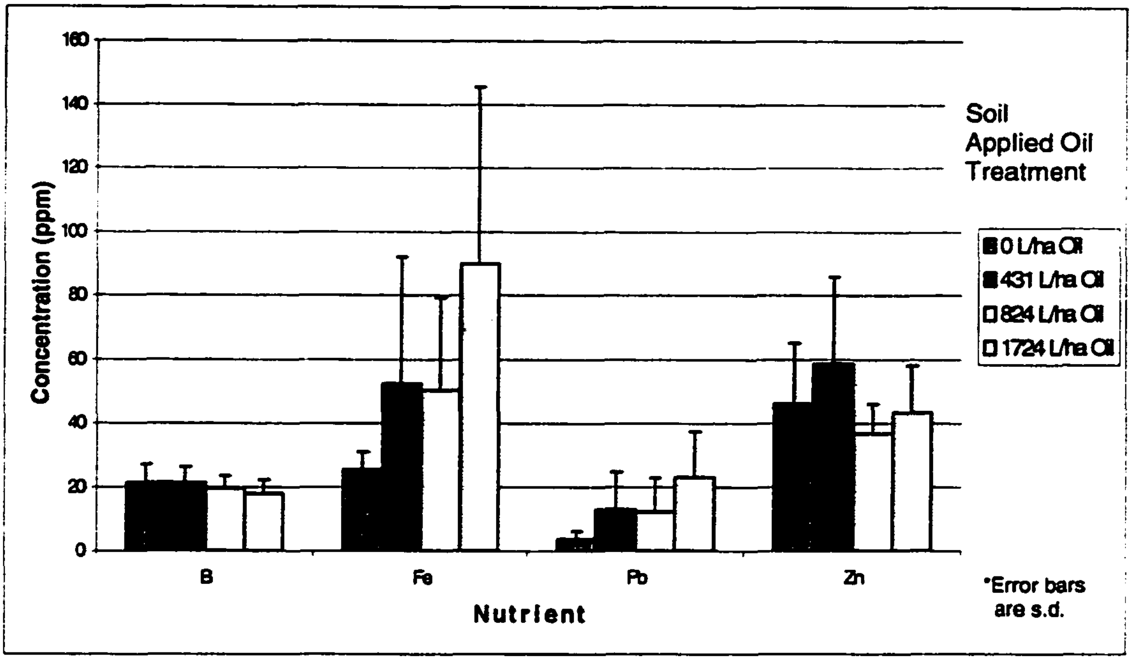

Figure 3.11 B, Fe, Pb, and Zn Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Oil.

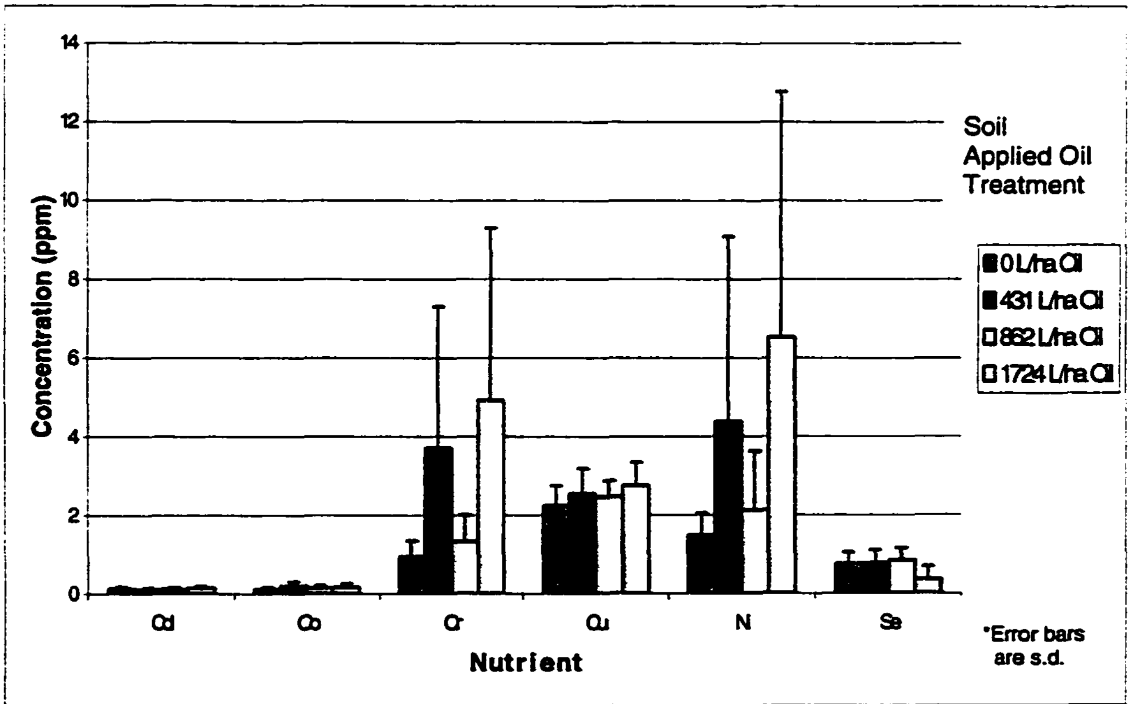

Figure 3.12 Cd, Co, Cr, Cu, Ni, and Se Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Oil.

of the seedlings. The interactions of oil with roots needs further research to positively prove that the oil is indeed inducing these changes within the plant.

3.3.3.2 Soil Hydrocarbon Analysis

The TPH analysis of the soils showed that the ABG decreased the hydrocarbon concentrations when added at high enough rates (Figure 3.13). In soils treated with 862 L/ha oil, ABG reduced TPH concentrations when added at rates of 1742 kg/ha (18201 ppm to 1438 ppm) and 862 kg/ha (18201 ppm to 3585 ppm). In soils treated with 1724 L/ha oil, ABG reduced concentrations when added at rates of 1724 kg/ha (18571 ppm to 574 ppm) and 862 kg/ha (18571 ppm to 915 ppm). A less dramatic decline in oil concentration occurred with the 431 kg/ha ABG treatments. As with the wetland soil study, high variability exists within treatments. Results of this study show that ABG has the ability to enhance the remediation of oil contaminated forest soils.

The application of the ABG did not seem to increase the odds of survival of the seedlings when exposed to high concentrations of oil in the soil. The health of the seedling and rate of bagasse applied to the soil was not significant ($p=0.3702$). By the time the ABG was added, the high concentrations of oil had already injured the seedling beyond recovery. If the ABG was applied sooner than 14 days after the oil was applied, results may have been different. If applied one to three days after oil spills and at appropriate rates equal to TPH soil concentrations, ABG may be able to prevent the death of some forest seedlings and other vegetation by preventing oil interference with root functions.

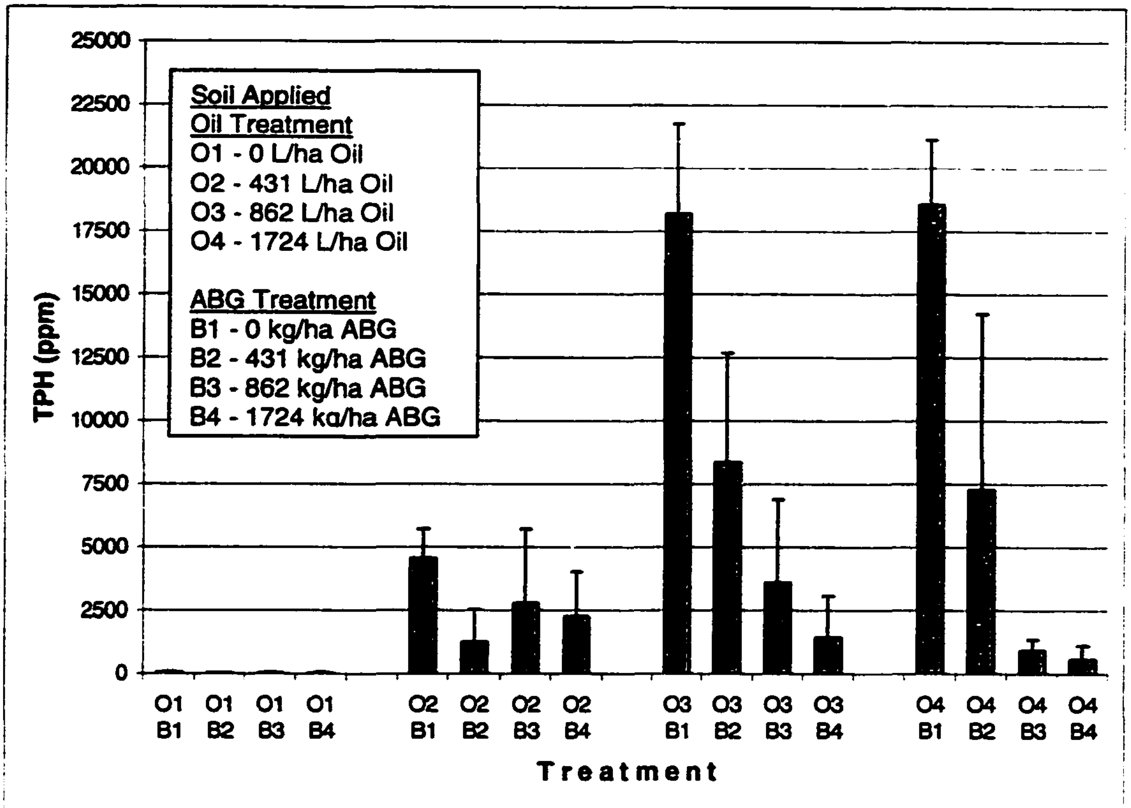

Figure 3.13 Soil Total Petroleum Hydrocarbon Concentration as a Result of Application of Ammoniated Bagasse (ABG) to Oil Contaminated Forest Soil.

The number of fine roots per tree is a function of the ecological and environmental conditions of the site. Most fine roots die in unfavorable conditions, but reform rapidly when conditions become more favorable. Obviously, oil spills on a site will degrade the ecological and environmental conditions. If oil contaminated forest soils are remediated as soon as possible after the spill, then the negative effects on vegetation may be reduced.

3.3.4 N:P Ratios

A common method to diagnose the health of plants is to examine the N:P ratio. The N:P ratios for loblolly pine can vary depending on the age of the tree. Studies by Allen (1987) and Bekele (1997) have found a needle N:P ratio range between 10 to 17 for semi-mature loblolly pines. A study by Wells *et al.* (1975) on a 16-year old loblolly pine stand reported needle N:P ratios ranging from 7.9 to 8.7. The needle N:P ratios for the seedlings in the greenhouse study ranged from 10.3 to 18.1, with the soil applied oil seedlings having the highest ratios (Table 3.7). The soil treated seedlings had higher N:P ratios because the seedlings had higher concentrations of N and lower concentrations of P compared to foliar oil treatments. There is no significant difference in the N:P ratios when comparing the pre-study seedlings and the foliar oil study seedlings. These ratios fall within the reported range of semi-mature loblolly pine N:P ratios. Therefore, using N:P ratios to diagnose the health and cause of death of the greenhouse seedlings may not prove useful.

3.4 Discussion and Conclusions

The results of the oil greenhouse study prove that crude oil does have a negative impact on loblolly pine seedlings and may have been the principle cause of death of the trees at the Cravens and Union Hill oil well blowout sites. Aerial spray of the oil at these sites saturated the needles of the seedlings immediately adjacent to the wells. As the foliar oil greenhouse studies demonstrated, when oil covers greater than 70% of the needle surface area the

Table 3.7 Mean Needle N:P Ratios for Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar and Soil Applied Oil*.

	Foliar Oil Pre-study								
Foliar Coverage	20%	30%	40%	50%	60%	70%	80%	90%	100%
N:P Ratio	11.7[a]	11.9[a]	14.5[b]	13.4[b]	14.6[b]	11.9[a]	11.6[a]	11.4[a]	10.3[a]
	Foliar Oil Study								
Foliar Coverage	0%	25%	50%	75%	100%				
N:P Ratio	14.3[b]	12.4[ab]	11.6[a]	11.3[a]	11.5[a]				
	Soil Applied Oil Study								
Soil Applied Oil	0 L/ha	431 L/ha	862 L/ha	1724 L/ha					
N:P Ratio	15.6[a]	18.1[a]	17.2[a]	16.8[a]					

*Means with the same letter within rows are not significantly different at α=0.05.

seedling will probably die. Nutrient analysis of these seedlings suggests that the photosynthetic pathway may be disturbed. This may be inferred from the decrease in Cu, Fe, Mg, Mn, and N concentrations in the seedlings that died. Previous studies (Baker, 1970) reported decreased photosynthetic rates primarily by physical interference with gaseous exchange and blocking of stomata. This conclusion, though, may be questioned because the effects of different treatments of foliar oil were not statistically significant for all of these nutrients. Also, age of the seedlings and environmental conditions within the greenhouse may have differed from the field conditions making it difficult to compare seedling reactions to the oil to mature tree reactions in the field. No direct evidence of changes in photosynthesis were assessed by this study.

Crude oil foliar applied to the seedlings may have reduced photosynthetic rates similar to the action of antitranspirants. Antitranspirants are chemicals often applied to plants to reduce transpiration rates and improve water balance.

This is achieved by causing stomatal closure or by coating the foliage with a film impermeable to water (Kozlowski *et al.*, 1991). The effects of these chemicals on photosynthesis vary with the compounds and the dosage used. If used improperly, antitranspirants can inhibit photosynthesis, causing stress and even death of the plant. In studies by Lee and Kozlowski (1974) and Davies and Kozlowski (1974), photosynthesis was reduced in angiosperms and gymnosperms as a result of stomatal plugging. Stomatal plugging occurred as the antitranspirant combined with waxes in stomatal pores to form impermeable plugs. This plugging was followed by altered metabolism, chlorosis, browning of leaves, and reduced leaf growth. These studies also showed that antitranspirants reduced photosynthesis by as much as 90% within red pine (*Pinus resinosa*). The stress and death of the loblolly pine seedlings may have been caused by a similar action due to the oil. These seedlings showed reduced concentrations of nutrients essential for photosynthesis, chlorosis, and browning of needles.

The time of year of oil application obviously has some effect on the reaction of the pine seedlings. The pre-study seedlings sprayed in early April took nearly two months to show signs of stress, while foliar oil seedlings sprayed in mid-May showed signs of stress two weeks after application. All seedlings receiving foliar applications of oil began to show signs of stress around the same time, early June. This may be due to seasonal growth effects and/or temperature interactions.

Photosynthetic activity of a plant is related to air temperature. As air temperatures increase, photosynthetic rates increase. This type of seasonal relationship is seen in loblolly pines. A study by McGregor and Kramer (1963) observed that beginning in February the rate of photosynthesis of loblolly pine increased slowly until April, then accelerated rapidly during the summer and declined during autumn and winter. Teskey and Will (1999) state that the optimum temperature for net photosynthesis in loblolly pines is between 25 and 30°C and that long-term exposure to high temperatures (35-40°C) had detrimental effects on photosynthetic capacity.

The average temperatures for April, May, and June in East Baton Rouge Parish are 20°C, 23°C, and 26°C, respectively (USDA, 1968). In April, when the pre-study seedlings were sprayed with oil no signs of stress were seen. At the time of application, photosynthetic rates would be slowly increasing. The foliar oil study seedlings sprayed in mid-May would have higher photosynthetic rates at the time of application as compared to the pre-study seedlings. These foliar oil seedlings exhibited signs of stress approximately two weeks after application, nearly the same time the pre-study seedlings began to show visual signs of stress. The temperature at which all seedlings receiving foliar applications of oil began to show signs of stress falls within the optimum range for net photosynthetic activity (25-30°C). As temperatures and photosynthetic rates increased, seedlings begin to exhibit signs of stress caused by the oil. During this period of increasing temperature, respiration rates would also be increasing. The lack of transpirational cooling caused by stomatal blockage by the oil may lead to excessive leaf temperatures, that would cause needle death and as a result decrease photosynthetic activity. The oil well blowouts at Cravens and Union Hill took place during the summer months and the contaminated trees showed immediate signs of stress.

A similar type of reaction to oils can be seen when using crop oil concentrates with herbicides. Crop oil concentrates (COCs) are a blend of non-phytotoxic (83-85%) petroleum or vegetable-based oil plus a surfactant (15-17%). The functions of this product are to reduce surface area tension between leaf surfaces and spray droplet, to promote herbicide penetration into leaves, and to prolong drying time to allow more herbicide to be absorbed (Iowa State University Extension, 2001). Plant age and size, relative humidity, soil moisture, and temperature are factors that affect the efficiency of COCs (University of Illinois Cooperative Extension Service, 1998). If COCs are applied when temperature and relative humidities are high, rates of transpiration and photosynthesis may decrease due to stomatal interference with gaseous exchange. As temperatures increase, seedlings treated with foliar applications of crude oil may exhibit similar reactions as seen with COCs.

Other physiological process, such as respiration or transpiration, may also have been affected by the oil applications. As temperatures increased, respiration rates may have exceeded photosynthetic rates resulting in stressing of the seedling. Stomatal plugging caused by the oil may also have caused transpiration rates to decrease resulting in increased temperatures within the seedlings (Baker, 1970).

A trend that was seen in the foliar oil treatments was that new shoot growth showed the first signs of stress. This new growth may be more susceptible to oil contamination because it lacks waxes that help to protect the needles. It can be concluded, though, that seedlings that have 50% or less needle surface area coverage by oil are likely to survive, but will show some signs of stress. These seedlings will have some needle chlorosis and wilting.

Oil added directly to the soil had detrimental effects on the pine seedlings. Some type of root interaction with the oil probably caused these effects. Oil which coats absorbing roots may potentially inhibit water and nutrient uptake resulting in drought-like reactions from the vegetation (i.e. needle wilting and chlorosis). The oil may also bind soil particles together impeding water and oxygen, which may affect nutrient availability to the plants. These conditions may mimic soil water deficits, which in turn may cause leaf water deficits. When leaf water deficits occur, reduction of leaf area and closing of stomata inhibits photosynthesis. This type of interference would directly affect growth and survival of the plant. Udo and Fayemi (1975) found that plants growing in oil contaminated soil had reduced growth rates and chlorotic leaves and were dehydrated, indicating soil water deficiencies. This type interaction of oil with absorbing roots and/or the soil caused of death of these seedlings.

The effects of an oil well blowout on surrounding vegetation may be predicted by sampling needles and performing nutrient analysis. Results of the greenhouse studies indicate that heavy metal concentration can be potentially used to indicate the degree of stress for the seedlings. As the seedlings in the studies became more stressed, they were no longer able in inhibit uptake of heavy metals (Cr, Ni, Pb, etc.). Seedlings that accumulated heavy metals eventually died, although death may have not been directly due to heavy metal contamination. Decreases in Cu, Fe, Mg, Mn, and N concentrations would indicate that the seedling is no longer able to sustain rates of photosynthesis needed to keep the tree healthy. This type of analysis would help responsible parties determine the exact environmental damage sustained by the surrounding vegetation. This would be valuable information when it comes to remediating a contaminated site.

The ammoniated bagasse was efficient in removing oil from the forest soil and reducing TPH concentration. If sufficient amounts of ABG are applied immediately after an oil spill, the negative effects on vegetation may have the potential to be reduced. The ABG would be a beneficial tool to use at oil contaminated forest sites. Further field studies with ABG and oil contaminated forest sites should continue. A study repeating the greenhouse study in a field situation would help to determine the effectiveness of ABG under natural environmental conditions. Also, testing ABG at smaller oil spill sites may help to assess its effectiveness at remediating forest soils during larger spill events.

CHAPTER 4

EFFECTS OF FOLIAR AND SOIL APPLIED BRINE ON TWO-YEAR OLD LOBLOLLY PINE (*PINUS TAEDA*) SEEDLINGS

4.1 Introduction

Brine is the primary byproduct of oil production and exploration. The American Geology Institute (1987) defines brine as warm to hot, highly saline waters containing Ca, Cl, K, and Na and minor amounts of other elements. When an oil well blowout occurs, most of the remediation efforts are geared toward cleaning up and removing the oil. In most blowouts, brine is also a concern because of its adverse effects on vegetation and wildlife. There has been much research focused on reclamation of agricultural land affected by waters high in sodium and the effects of road de-icing salts on vegetation, but little information exists in the literature regarding reclamation of forested lands that have been contaminated with brine (Webster *et al.*, 1983). The effects of concentrated brine on forest tree species have never been investigated. In general, the effects of sodium chloride (NaCl) on vegetation can be considered when the effects of brine are investigated.

It has been shown that salt adversely affects plant growth in two ways. First, high concentrations of specific ions in salt may be toxic and induce physiological disorders (Treacy, 1984). Second, soluble salts lower the water potential in soil and restrict water uptake by the roots (Bernstein and Hayward, 1958). Salt affected plants are stunted, have darker foliage, and have thicker and more succulent leaves (Jennings, 1976). A common visual effect of salt stress is leaf or needle burn. Bernstein and Hayward (1958) describe leaf burn as tan or brown necrotic lesions sharply delineated from adjacent healthy green tissue.

Aerial salts, such as brine spray from an oil well blowout, injure vegetation by either foliar absorption or soil salinization. Bernstein (1975) states rates of salt absorption by foliage are often 100 times that of roots. Injury symptoms caused by foliar absorption of road de-icing salts included damage to dormant terminal buds, necrotic spots, burning and fall of leaves, delay in leafing, and petiole damage (Feder, 1977). Studies by Auchmoody and Walters (1988 and 1989) suggest that residual phytotoxicity of brine is short lived. This conclusion indicates that brine may not be an obstacle to overcome when remediating oil well blowout sites.

The published literature has not adequately addressed impacts of brine on forest vegetation, its residual toxicity, or vegetative recolonization patterns on brine killed areas (Auchmoody and Walters, 1988). At oil well blowout sites in Cravens and Union Hill, LA, loblolly (*Pinus taeda*) and longleaf (*Pinus palustris*) pine forest stands adjacent to the wells were severely impacted. This may have been due to oil, brine, gas, or a combination of these. The goal of this study was to determine the effects of foliar and soil applied brine to two-year old loblolly pine seedlings in a greenhouse experiment using nutrient concentration analyses.

4.2 Materials and Methods

The United States Forest Service donated approximately 1000 one-year old pine seedlings for this study. These seedlings were planted in March 1999 in seedling pots filled with the A and B horizons of a Malbis silt loam (Fine-loamy, siliceous, subactive, thermic Plinthic Paleudult) in order to imitate forest soil conditions. The seedlings were watered with de-ionized water every other watering in order to reduce salt buildup in the soil. The seedlings grew for one year under monitored greenhouse conditions before any treatments were applied.

4.2.1 Foliar Brine Study

A foliar brine study was initiated in May 2000. Brine from the Cravens, Louisiana oil well was obtained and the electrical conductivity was measured to be 60 mS/cm (milliSiemens per centimeter). Seedlings were randomly selected from the greenhouse population and assigned a treatment. The brine was foliar applied using needle surface area coverages of 0, 25, 50, 75, and 100%, with 15 replications of each treatment for a total of 75 seedlings. The needle surface area coverages were determined using the same method as in Chapter 3, Section 3.2.1. The height and diameter of each seedling were measured before the treatments were applied. The seedlings were sprayed with brine on May 15 and 22, 2000.

The seedlings were monitored and watered with de-ionized water every other watering to reduce salt buildup in the soil. The seedlings were harvested in October 2000. The height and diameter were once again measured before the seedlings were separated into needles, stems, and boles. A visual health rating was assigned to each seedling before harvesting. A numbering system from one to five was used, with one being a healthy seedling showing no signs of stress and five being a seedling with all needles brown and assumed to be dead. Nutrient concentration analysis for each part of each seedling was performed in order to determine the cause(s) of stress and death of any seedlings as a result of foliar brine applications. The seedlings were analyzed for Al (aluminum), As (arsenic), B (boron) Ca (calcium), Cd (cadmium), Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), K (potassium), Mg (magnesium), Mn (manganese), Mo (molybdenum), N (nitrogen), Na (sodium), Ni (nickel), P (phosphorus), Pb (lead), S (sulfur), Si (silicon), Se (selenium), and Zn (zinc) using the same modified block digestion technique (Bell, 2000) used in the oil greenhouse studies (Chapter 3) and a Perkin-Elmer ICAP analyzer.

4.2.2 Soil Brine Application Study

A study investigating the effects of soil applied brine was initiated in June 2000. Seedlings were randomly selected from the greenhouse population and assigned a treatment. Brine from the Cravens, LA oil well was applied directly to the soil to achieve soil conductivities of 0, 2, 4, 8, 16, and 32 mS/cm, with five replications of each treatment. Brine was added to the soil surface and allowed to infiltrate into the subsurface. The height and diameter of each seedling were measured before any treatments were applied. The seedlings were monitored and watered with de-ionized water every other watering to reduce salt buildup in the soil. The seedlings were harvested in October 2000. The height and diameter were once again measured before the seedlings were separated into needles, stems, and boles. The same visual health rating system used in the foliar brine study was also used in this study. Nutrient concentration analysis for each part of each seedling was performed in order to determine the cause(s) of stress and death of any seedlings as a result of soil applied brine applications. The seedlings were analyzed for Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, S, Si, Se, and Zn using the same modified block digestion technique (Bell, 2000) as in the foliar brine study and a Perkin-Elmer ICAP analyzer.

4.3 Results

Many studies investigating the effects of single or multiple stresses on plants often focus on the root-shoot response to the stress(es). Responses in these parts of the plant help describe the whole-plant response. In the greenhouse studies, nutrient analysis of needles, stems, and boles took place to investigate the seedling response to brine stress. Only the needle nutrient concentration results are presented and discussed here, as there were no unusual results from the stem and bole data that require discussion. Tables A7 through A10 present the stem and bole nutrient concentrations for the brine studies.

4.3.1 Foliar Brine Study

The results of the foliar brine study were interesting. There was expected to be some negative reaction of the pine seedlings due to the application of brine. Surprisingly, none of the seedlings died and few signs of stress were observed as a result of the foliar brine applications (Table 4.1). The only sign of stress was some needle burn on the tips of some of the 100% treated seedlings. This

Table 4.1 Mean Health Ratings, Height Change, and Diameter Change of Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar Applied Brine.

Needle Surface Area Covered with Brine	Mean Health Rating*	Mean Height Change (cm)	Mean Diameter Change (mm)
0%	1.3^{a}	0.9^{b}	4.58^{ab}
25%	1.1^{a}	1.5^{b}	5.68^{a}
50%	1.1^{a}	2.1^{ab}	5.06^{ab}
75%	1.3^{a}	4.0^{a}	3.38^{bc}
100%	1.4^{a}	2.0^{ab}	2.28^{c}

*Ratings: 1 (healthy, no visual signs of stress); 2 (slight visual signs of stress, few chlorotic and wilting needles); 3 (moderate signs of stress, chlorotic needles, wilting needles, some premature needle loss); 4 (severe signs of stress, chlorotic and necrotic needles, extreme wilting, premature needle loss); 5 (seedling is dead, all needles brown).
Means with the same letter within columns are not significantly different at $\alpha=0.05$.

occurred approximately two to three weeks after treatment. No other signs of stress were seen on these or any other treatments.

Statistical analysis reported that height ($p=0.0081$) and diameter (<0.0001) changes were significant for treatments. Seedlings treated with foliar applications of brine had greater increases in height change when compared to the control seedlings. Seedlings treated with 75% and 100% foliar brine had smaller changes in diameter compared to the other treatments. This may be due to brine stress causing a reduction in the diameter growth rate.

Table 4.2 Mean Needle Nutrient Analysis of Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar Applied Brine (% foliar coverage).*

Nutrient	0%	25%	50%	75%	100%
Al[t]	234	226	283	296	278
B	26.8	17.7	22.4	21.1	24.3
Ca	1488	1327	1655	1361	1908
Cd	0.073	0.104	0.085	0.023	0.107
Co[t]	0.124	0.105	0.159	0.123	0.151
Cr[t]	1.29	1.11	1.27	0.95	1.17
Cu[t]	2.76	1.94	2.41	3.39	1.91
Fe	28.4	21.3	25.1	23.6	35.9
K	1414	1717	1759	1634	1629
Mg	562	551	610	542	662
Mn	372	301	342	319	400
N	10903	9377	10084	8991	10279
Na	4681	3043	3681	3916	4603
Ni[t]	1.13	1.25	1.46	0.80	1.16
P	643	508	572	481	538
Pb[t]	4.58	3.97	4.99	6.73	5.47
S	481	417	459	410	475
Se	3.29	0.85	2.25	2.29	1.55
Si	102	76	107	83	99
Zn	36.7	29.5	33.1	34.6	44.6

*Mean values are parts per million (ppm).
[t]Treatment effects are not significant at α=0.05.

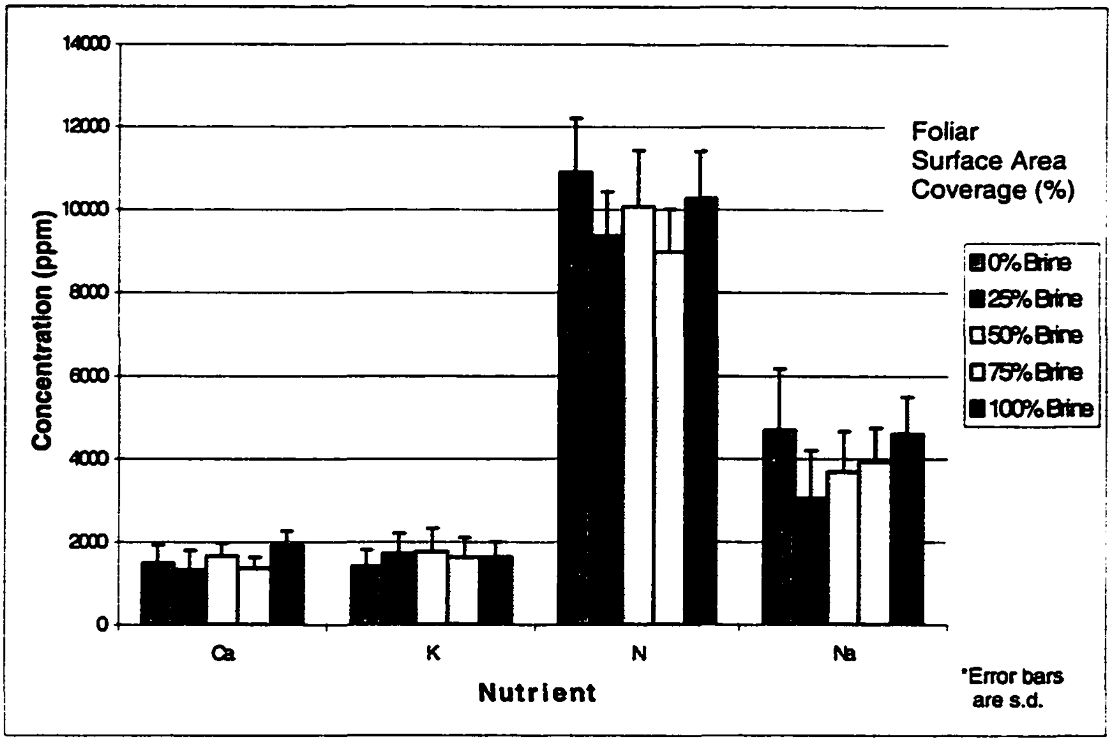

Figure 4.1 Ca, K, N, and Na Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Brine.

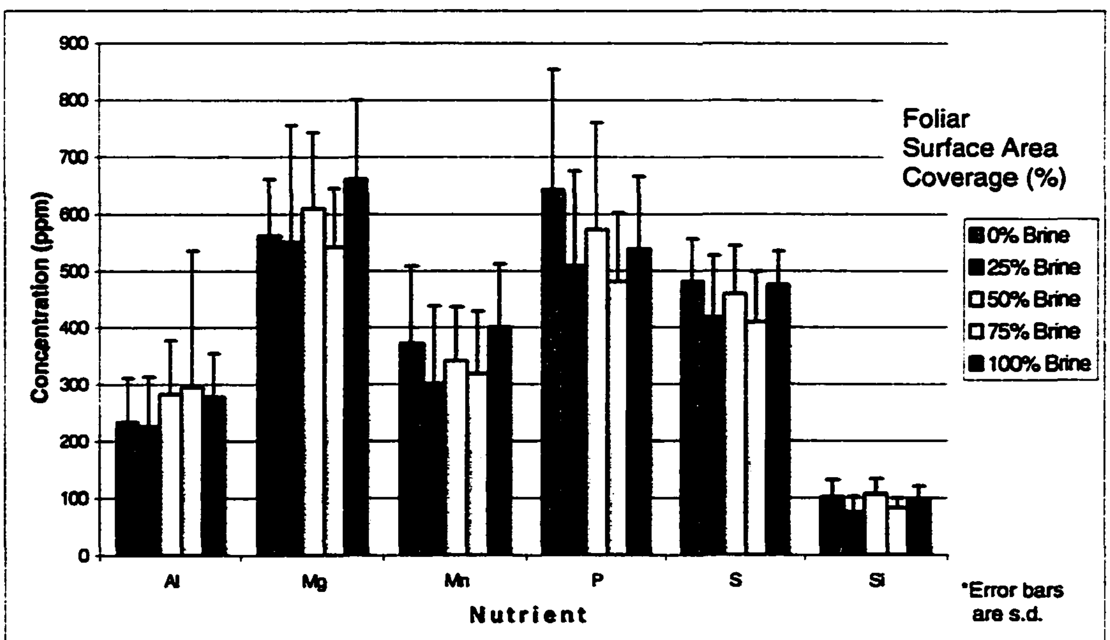

Figure 4.2 Al, Mg, Mn, P, S, and Si Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Brine.

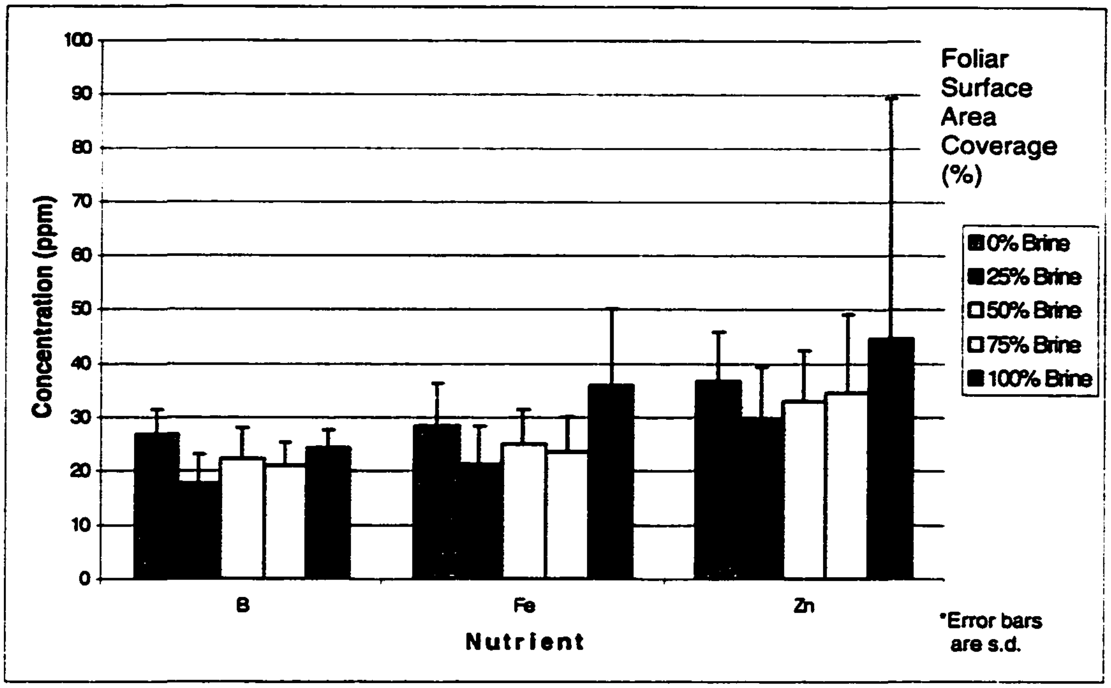

Figure 4.3 B, Fe, and Zn Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Brine.

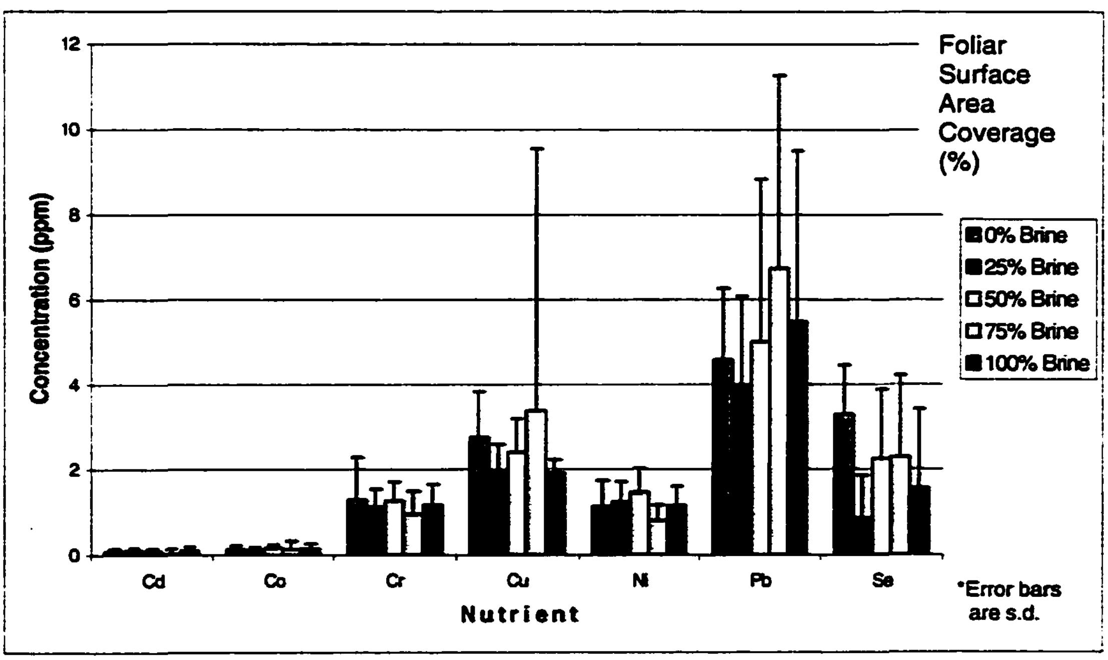

Figure 4.4 Cd, Co, Cr, Cu, Ni, Pb, and Se Needle Concentrations of Two-year Old Loblolly Pine in Response to Foliar Applied Brine.

Since none of the seedlings died or showed signs of stress it was difficult to identify significant trends in the nutrient analysis (Table 4.2, Figures 4.1-4.4). A trend was detected for Fe, Mg, and Mn. The concentrations of these nutrients increased from the 75% treatments to the 100% treatments. Treatment effects for these nutrients were significant. These nutrients play important roles in the photosynthetic process. Increases in these nutrients in the 100% treated seedlings may have resulted from the seedlings allocating more resources in order to overcome what little stress was caused by the brine (Kozlowski *et al.*, 1991). An increase in the rate of photosynthesis would account for the increased concentrations of Fe, Mg, and Mn. Nitrogen concentrations showed an overall decrease with increasing surface area coverage. From these results it can be suggested that brine accumulation on pine needles of the trees surrounding the oil wells may not have caused the seedlings to die.

4.3.2 Soil Brine Application Study

The results of brine added directly to the soil were as expected. Most vegetation can tolerate soil salinity conditions up to 4 mS/cm (Bohn *et al.*, 1985). Salt concentrations greater than this will usually result in death of the plant due to osmotic changes that restrict water and nutrient uptake from the soil to the roots. The effect of high salinities on plants appears to be energy diversion from growth processes in order to maintain the osmotic potential (Bohn *et al.*, 1985). This decrease in growth rate was observed from the greenhouse study (Table 4.3). Seedlings treated with soil concentrations greater than 4 mS/cm brine died and seedlings treated with soil concentrations equal to or less than 4 mS/cm brine survived.

Seedlings treated with 32 mS/cm brine began to show signs of stress within one week after application. Signs of stress included needle chlorosis and wilting. All of these seedlings died approximately three weeks after treatment. Seedlings treated with 16 mS/cm brine showed similar signs of stress one to two weeks after application. These seedlings died approximately one to two weeks after the 32 mS/cm treated seedlings. The 8 mS/cm brine treated seedlings also had needle wilting and chlorosis, but only two of the five replications died. The other three replications showed moderate signs of stress. The 4 mS/cm and 2 mS/cm treated seedlings showed little or no signs of stress, as expected. Some needle wilting was the only visual sign of stress observed. None of these seedlings died.

For treatments, changes in height were statistically significant ($p=0.0128$), while changes in diameter were not significant ($p=0.2377$). Seedlings treated with 0, 2, and 4 mS/cm brine had greater increases in height and diameter compared to the 8, 16, and 32 mS/cm treated seedlings. This would be expected as high salt concentrations in the soil result in drought-like reactions from the seedlings causing a stunting of growth.

Nutrient analysis of the seedlings showed some noticeable trends (Table 4.4, Figures 4.5-4.8). Seedlings that were stressed (8, 16, 32 mS/cm brine treated seedlings) had increased concentrations of Cu, Fe, K, Mg, Mn, Na, P, S,

Table 4.3 Mean Health Ratings, Height Change, and Diameter Change of Greenhouse Grown Two-year Old Loblolly Pine in Response to Soil Applied Brine.

Brine Treatment	Mean Rating*	Mean Height Change (cm)	Mean Diameter Change (mm)
0 mS/cm	1.8^{b}	2.0^{ab}	1.73^{a}
2 mS/cm	2.0^{b}	8.6^{a}	1.61^{a}
4 mS/cm	1.4^{b}	2.0^{ab}	2.45^{a}
8 mS/cm	3.6^{a}	0.6^{b}	1.41^{a}
16 mS/cm	5.0^{a}	1.2^{b}	0.94^{a}
32 mS/cm	5.0^{a}	0.4^{b}	0.75^{a}

*Ratings: 1 (healthy, no visual signs of stress); 2 (slight visual signs of stress, few chlorotic and wilting needles); 3 (moderate signs of stress, chlorotic needles, wilting needles, some premature needle loss); 4 (severe signs of stress, chlorotic and necrotic needles, extreme wilting, premature needle loss); 5 (seedling is dead, all needles brown).
Means with the same letters within columns are not significantly different at α=0.05.

and Se when compared to unstressed seedlings. Sodium concentrations increased with treatment, as expected. The increased concentrations of Cu, Fe, Mg, and Mn may be due to seedlings allocating more resources to photosynthesis as they become stressed. It is obvious that the seedlings died due to highly saline soils caused by brine contamination.

4.3.3 N:P Ratios

The N:P ratios for the brine affected seedlings were slightly higher than reported values for semi-mature loblolly pine (Table 4.5). Ratios ranged from 9.3

Table 4.4 Mean Needle Nutrient Analysis of Greenhouse Grown Two-year Old Loblolly Pine in Response to Soil Applied Brine.*

Nutrient	0 mS/cm	2 mS/cm	4 mS/cm	8 mS/cm	16 mS/cm	32 mS/cm
Al[t]	270	229	255	231	238	273
B	24.2	51.7	60.4	171.6	105.3	83.7
Ca	1508	1323	1622	1615	2049	2781
Cd	0.081	0.069	0.089	0.121	0.183	0.186
Co	0.152	0.147	0.268	0.177	0.307	0.376
Cr	1.20	1.35	0.88	1.43	0.79	0.81
Cu	2.29	2.16	2.37	2.86	2.82	3.59
Fe	26.9	21.5	22.8	38.9	40.6	26.5
K	2211	1670	1764	2906	2715	3405
Mg	716	648	755	756	880	1060
Mn[t]	375	314	420	358	433	508
N	10814	11414	10636	11176	12598	11428
Na	3752	5869	5408	8860	10822	12427
Ni[t]	1.05	1.35	1.59	1.41	1.77	1.51
P	459	705	785	564	922	1225
Pb[t]	13.7	8.1	22.5	5.3	7.5	7.1
S	599	613	586	669	708	815
Se[t]	0.851	0.867	0.840	0.964	0.923	0.993
Si[t]	112	116	114	117	114	146
Zn	29.9	35.5	30.6	28.3	44.9	39.3

*Mean values are parts per million.
[t]Treatment effects are not significant at α=0.05.

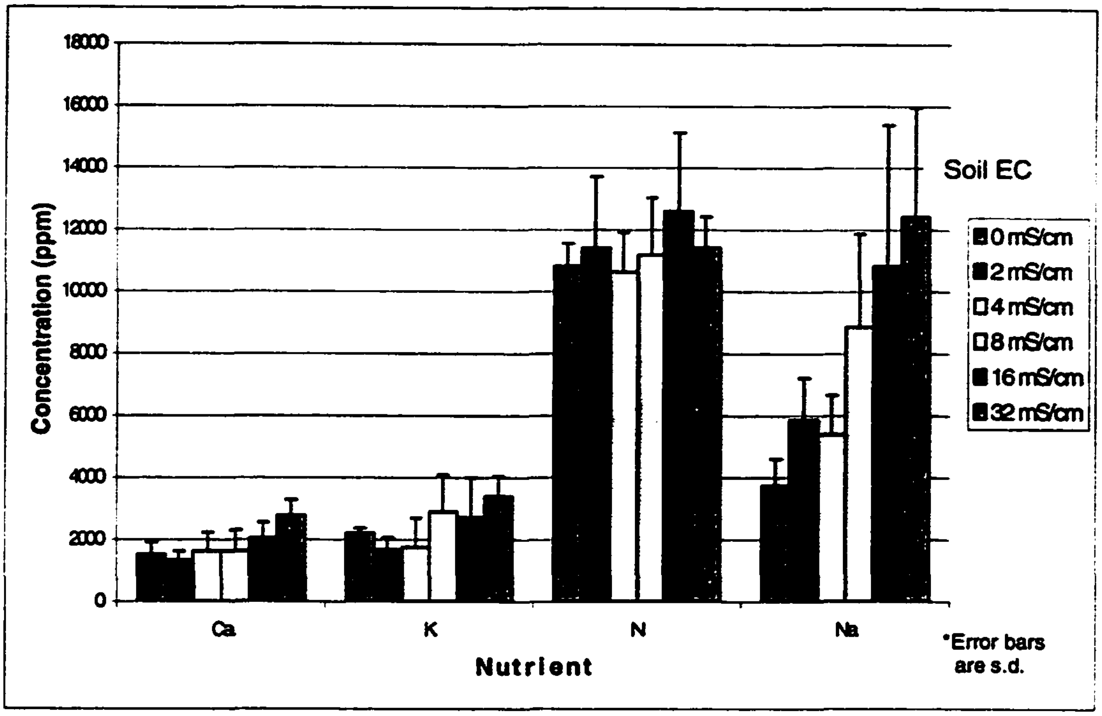

Figure 4.5 Ca, K, N, and Na Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Brine.

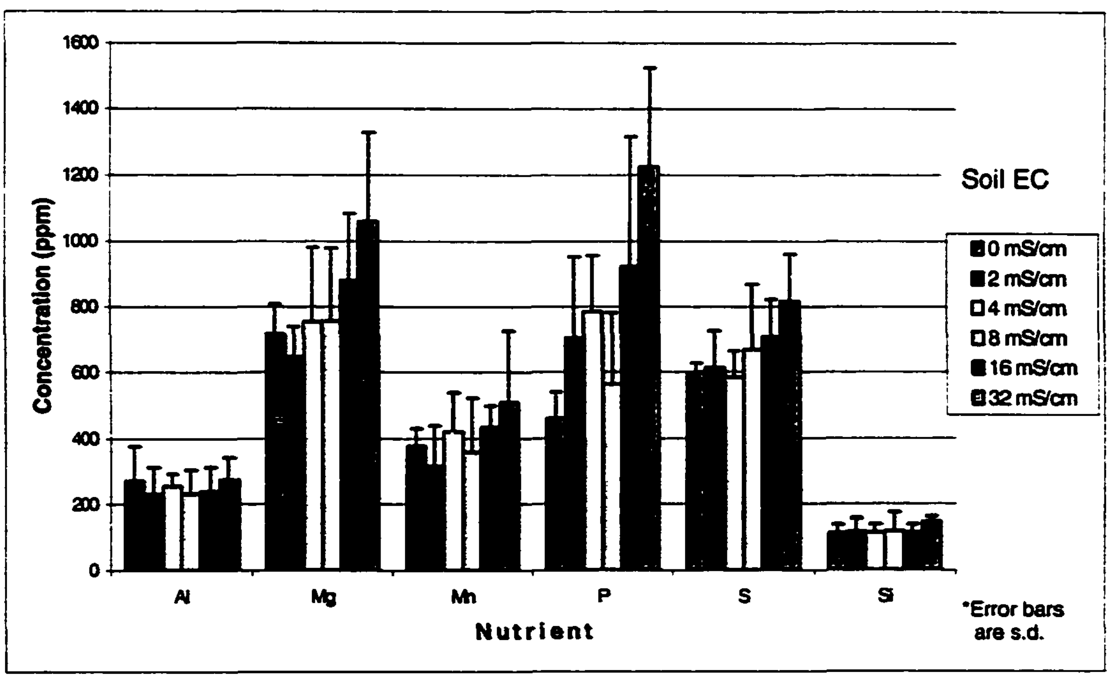

Figure 4.6 Al, Mg, Mn, P, S, and Si Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Brine.

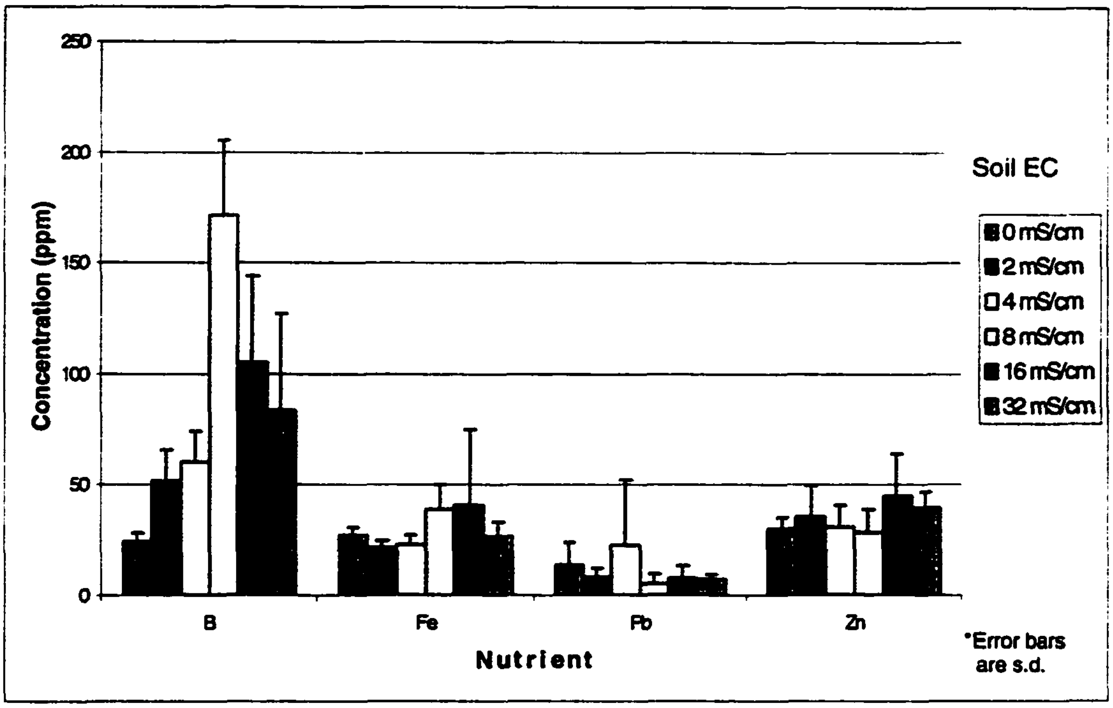

Figure 4.7 B, Fe, Pb, and Zn Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Oil.

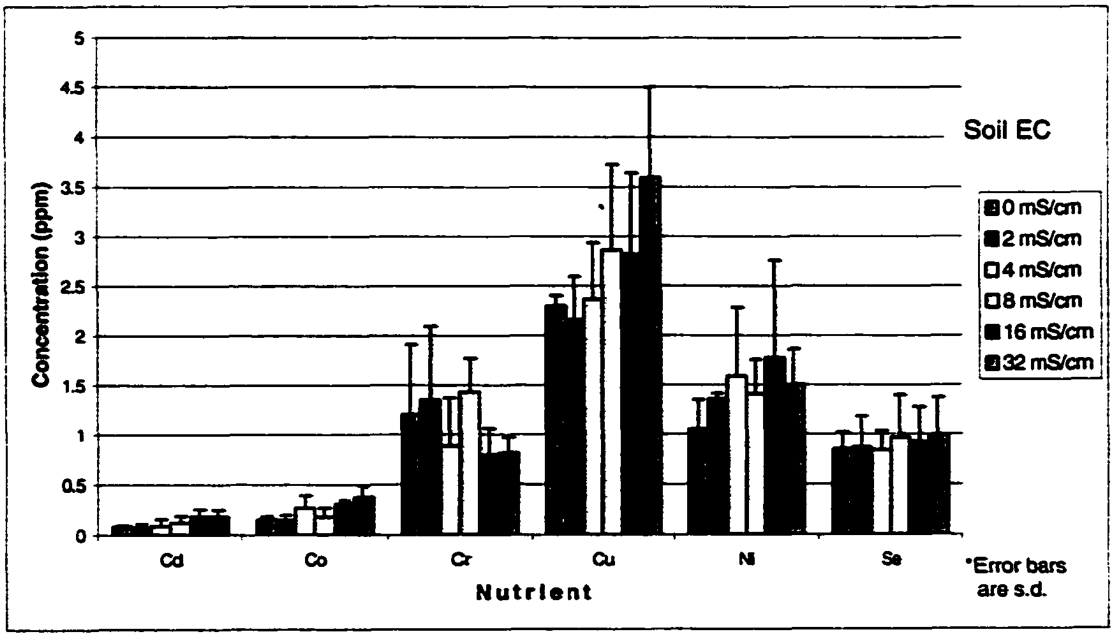

Figure 4.8 Cd, Co, Cr, Cu, Ni, and Se Needle Concentrations of Two-year Old Loblolly Pine in Response to Soil Applied Brine.

to 23.6. The N:P ratios for the different foliar brine treatments were not significantly different from each other. The control treatment in the soil applied brine study had the highest N:P ratio due to a low concentration of P. The ratios of the soil applied brine treatments decrease with treatment due to higher P concentrations. The 32 mS/cm treatment had the lowest N:P ratio due to a high P concentration. Since these N:P ratios are not significantly different than reported values found in the literature, using these ratios may not be useful in determining the health or cause of stress or death of the seedling. The nutritional effects of salinity on plants are poorly understood (Bohn *et al.*, 1985), therefore it is difficult to determine how the changes in N and P are affected by the brine concentrations.

Table 4.5 Mean Needle N:P Ratios for Greenhouse Grown Two-year Old Loblolly Pine in Response to Foliar and Soil Applied Brine.*

Foliar Brine Study					
Foliar Coverage	0%	25%	50%	75%	100%
N:P Ratio	16.9[a]	18.5[a]	17.6[a]	18.7[a]	19.1[a]

Soil Applied Brine Study						
Soil Salinity	0 mS/cm	2 mS/cm	4 mS/cm	8 mS/cm	16 mS/cm	32 mS/cm
N:P Ratio	23.6[a]	16.2[b]	13.6[b]	19.8[ab]	13.7[b]	9.3[c]

*Needle N:P ratios for semi-mature loblolly pine range from 10 to 17 (Bekele, 1997 and Allen, 1987).
Means with same letter within rows are not significantly different at α=0.05.

4.4 Discussion and Conclusions

From the results of these studies, it may be suggested that foliar brine did not kill the pine trees in the direct vicinity of the oil well blowouts. Foliar applied brine had little or no effect on the seedlings in the greenhouse. Soil applied brine, on the other hand, had a great effect on the seedlings when concentrations exceeded 4 mS/cm. The greenhouse results may not apply, though, to mature trees in the field as age and environmental conditions are not similar. As a result, it is difficult to state whether the brine from the oil well had no adverse effect on the surrounding pine stand. Saturating a tree with brine for eight straight days, as at the Cravens site, may have caused the tree stress and eventually led to their death.

It seems from the foliar brine greenhouse study that the residual phytotoxicity of a one time application of brine is short lived. This supports a similar conclusion made in studies by Auchmoody and Walters (1988). In their studies they found that when an Allegheny hardwood stand was contaminated with brine, declines in health and growth rate of tree occurred within one month of contamination. Six months after the brine spill, the trees recovered and regeneration at the site occurred. Aerial dispersion of brine at blowout sites may have little or no effect on surrounding vegetation, unless brine infiltrates the soil and causes high soil salinities (> 4mS/cm) at which vegetation cannot survive.

Upland soil samples collected by the environmental consulting firm contracted by the owners of the oil well at the Cravens site after the oil well blowout showed electrical conductivities to be less than 1 mS/cm (C-K Associates, Inc., 1999). At this blowout site the soil brine concentrations were not high enough to warrant remediation efforts, as the concentrations were reduced by natural precipitation events. Therefore, it may be concluded that the oil or the gas from the blowout sites was the primary cause of death of the pine seedlings.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Oil and brine spills, such as the one at Cravens, Louisiana, will continue as long as exploration and production activities take place in Louisiana. The ecological damage caused by these spills will vary with site and environmental conditions. Forest and wetland contaminated areas pose a unique situation for oil and brine remediation and restoration efforts. Traditional remediation techniques employed in these environments have the potential to do more damage than the oil or brine. Alternative remediation techniques, such as using ammoniated bagasse, may help to remediate a site with potentially less impact to the environment.

Results of the wetland and greenhouse studies investigating the remediation of oil contaminated wetland and forest soils using ABG proved questionable. In the wetland field study, there was no statistically proven outcome that the ABG promoted decreases in TPH of the contaminated wetland soil. When ABG was applied at rates of 200 kg/ha to oil contaminated wetland soil, TPH concentrations seemed to decrease, but the high spatial variation of oil within the wetland makes this difficult to prove. Instead, the TPH decrease may have been the result of changing environmental conditions, such as water level, at the site. Applications of ABG at this site at rates higher than 200 kg/ha may be effective at remediating the soil. Larger experimental plots and a greater number of plots are needed to confirm potential benefits of ABG.

In the forest soil greenhouse study, applications of ABG to the contaminated forest soils proved more reliable compared to the wetland soil results. The ABG substantially reduced TPH concentrations (18571 ppm to 574 ppm) when applied at rates of 1725 kg/ha. If applied to the soil immediately after oil spills, ABG may absorb oil and help to prevent the death of some vegetation by minimizing oil interference with root functions. The timing of application of ABG to soils needs to be investigated because the effects of oil on the tree roots may be immediate and irreversible.

The use of ABG to remediate the oil contaminated wetland soils at the Cravens blowout could be the first step to restore the wetland. The hydrology, which is the most important component of wetland restoration, was minimally affected by the blowout. There has been a reduction in brine concentration in the soil (430 µS/m to 236 µS/m) and it is no longer considered a deterrent to restoration. Therefore, restoration efforts should focus on the removal of residual oil from the soil by using ABG and revegetate the site using selected wetland species. After oil concentrations have decreased to an acceptable level, a seed bank with selected natural wetland species can be introduced.

The greenhouse studies investigating the effects of oil and brine on two-year old loblolly pine had some interesting results. The most surprising finding was that none of the pre-study seedlings receiving a single foliar application of oil in April showed any signs of stress until early June. The seedlings treated with foliar applications of oil in mid-May showed signs of stress at nearly the same time in June that the pre-study seedlings began to stress. As temperatures and photosynthetic activities of the seedlings increased, visual signs of stress also increased. Although, signs of stress may have been cause by a decrease in transpiration due to oil blockage of stomata that would cause an increase in needle temperature. This increase in needle temperature may cause the needles to stress and as a result decrease rates of photosynthesis.

Nutrient analyses of the seedlings suggest that the oil may be inhibiting the photosynthetic process, although this was not statistically proven. Reduced concentrations of nutrients essential to the photosynthetic process (Cu, Fe, Mg, Mn, and N) were found in the seedlings that showed severe signs of stress and/or died. These decreases may have been caused by physical interference with gaseous exchange and stomatal plugging or the oil entering the needles and causing cells to be damaged by destroying the cell membrane. Visual signs of stress seen in the greenhouse seedlings included chlorotic needles, wilting needles, and browning of needles. These signs were also seen in previous studies investigating stomatal plugging caused by antitranspirants. The results suggest that the seedlings may have stressed and/or died as a result of stomatal interference with gaseous exchange, although this is based on the previous antitranspirant studies, not actual results from this study. This is an area of this study that needs further research to substantiate these conclusions.

Seedlings that received needle surface area coverages of oil greater than 70% showed the most severe signs of stress and died. Therefore, it may be concluded that the LD_{50} for two-year old loblolly pine receiving foliar oil applications of oil is approximately 70% foliar surface area coverage. Seedlings that receive this extent of oil contamination may show signs of stress and will probably die.

Seedlings receiving foliar oil coverages of 60% or less may also show signs of stress but might have a greater chance of survival.

The potential of surviving foliar oil contamination may also depend upon the time of year. All seedlings receiving foliar applications of oil began to show signs of stress around the same time of year, regardless of the time of year they were sprayed with oil. It seems that as the trees reached optimum temperatures for net photosynthesis (25-30°C) they became more susceptible to oil phytotoxicity. Seedlings contaminated with oil in the cooler months of the year, when photosynthetic rates are lower, may have a better chance of surviving the phytotoxic effects of oil compared to those seedlings contaminated when temperatures are higher. This may help to explain, in part, the rapid signs of stress seen at the Cravens blowout, which took place in August when temperatures in Louisiana are near their highest for the year.

Oil added directly to the soil had a rapid and detrimental effect on the pine seedlings grown in the greenhouse. Visual signs of stress observed included needle chlorosis, needle wilting, premature loss of needles, and death. These signs of stress may have been due to inhibition of water and nutrient uptake caused by the oil. Oil likely coated the absorbing roots of the seedlings, which would prevent water and nutrient uptake and result in drought-like reactions from the seedlings. The oil may also bind soil particles together impeding water and oxygen, which may also affect plant nutrient availability. Seedlings treated with 431 L/ha of oil were able to survive and showed minimal signs of stress, while those seedlings treated with greater than 862 L/ha of oil died. It is unlikely, though, that the upland soil oil concentrations at the Cravens blowout were high enough to cause the trees to die. TPH results of upland soil samples taken by C-K Associates, Inc. after the blowout were less than 100 ppm (C-K Associates, Inc., 1999). This soil concentration would be less than 100 L/ha oil added directly to the soil.

Single applications of foliar brine had little or no effect on the seedlings. This suggests that the residual phytotoxicity of brine is short lived and that aerial spray of brine at the Cravens blowout may not have been the cause of stress and/or death of the loblolly pines. The only sign of stress observed in the greenhouse was slight needle burn on the needle tips of some of the 100% trees. As expected, the brine applied directly to the soil killed the trees when soil salinities were greater than 4 mS/cm. At the Cravens blowout, upland soil brine concentrations were not high enough to cause saline soils and kill the trees, even though the blowout lasted eight days.

Greenhouse study results suggest that the trees impacted by the Cravens oil well blowout stressed and/or died as a result of foliar oil contamination. Loblolly pines immediately adjacent to the well probably received oil coverages greater than 70%, which would cause stress for the trees and they would eventually die. The time of year of the blowout, during August, may also have accelerated the phytotoxic effects of the oil due to decreased transpiration rates causing higher needle temperatures. Trees further away from the oil well probably received oil coverages of less than 70%, and as a result survived the contamination, but not without some stress. Needle nutrient concentration analyses will help to determine the impact of oil contamination on trees in the future, but more work needs to be performed investigating the action of oils on needles and roots. Further studies may use these results as a basis for further investigation of the effects of crude oil on trees and other vegetation.

During the course of the greenhouse studies, it was difficult to find baseline nutrient concentrations for two-year old loblolly pine. Information was available for most of the macro- and micronutrients for different aged trees (Table A1), but not for other elements, such as Al, Cd, Co, Cr, Ni, Pb, Se, and Si. Using the controls for each of the greenhouse studies, the concentrations of these elements were determined for needles, stems, and boles (Table A2). Comparing available data to the greenhouse data collected in these studies showed similar concentrations for macro- and micronutrients, with some differences due to age of tree when harvested. Nutrient concentration analyses of the controls in this study provide a baseline for 20 elements found in two-year old loblolly pine. This analysis is information that will be beneficial to both foresters and researchers alike.

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APPENDIX: BOLE AND STEM NUTRIENT ANALYSIS DATA FOR OIL AND BRINE TREATED TWO-YEAR OLD LOBLOLLY PINE (*PINUS TAEDA*)

Table A1. Mean Nutrient Concentrations for Loblolly Pine (*Pinus taeda*) Foliage.

Nutrient	Critical Level (ppm)
B	16-45[a]
Ca	1200-1400[b]
Cu	5-22[a]
Fe	30-88[c]
K	2500-3000[b]
Mg	700[b]
Mn	306-392[a]
Mo	N/A
N	11000[b]
P	900-1000[b]
S	500-800[c]
Zn	12-21[d]

[a]For four-year loblolly pine plantation, TVA (1968)
[b]For semi-mature loblolly pine, Allen (1987)
[c]For one-year old loblolly pine, Bekele (1997)
[d]For one-year old greenhouse grown loblolly pine, Coultas *et al.* (1991)

Table A2. Mean Nutrient Concentrations for Two-year Old Loblolly Pine.[a]

Nutrient	Needle	Bole	Stem
Al	251	113	118
B	25.1	10.2	15.1
Ca	1671	1581	1526
Cd	0.128	0.227	0.222
Co	0.144	0.079	0.108
Cr	1.01	1.05	1.02
Cu	2.435	2.424	2.969
Fe	30.7	45.2	36.9
K	1860	597	1097
Mg	674	451	491
Mn	314	144	161
N	10960	4330	5450
Na	4737	2225	3135
Ni	1.321	0.825	0.993
P	686	295	449
Pb	6.46	2.65	4.48
S	564	266	345
Se	1.347	1.239	1.333
Si	201	162	185
Zn	43.2	29.6	40.1

[a]Mean values are parts per million (ppm).

Table A3. Mean Bole Nutrient Analysis for Two-year Old Loblolly Pine in Response to Foliar Applied Oil (expressed in ppm).

Nutrient	0%	25%	50%	75%	100%
Al	161	154	178	152	144
B	16.38	12.95	11.56	11.75	8.93
Ca	1844	1645	1726	1637	1546
Cd	0.319	0.248	0.258	0.298	0.235
Co	0.133	0.122	0.145	0.126	0.195
Cr	0.995	0.980	1.031	1.140	0.942
Cu	2.704	2.218	2.014	2.101	1.979
Fe	69.4	52.7	82.7	157.2	81.2
K	797	814	816	762	707
Mg	583	468	546	488	469
Mn	198	175	189	185	156
N	4658	2926	3212	2930	3502
Na	3866	2234	2334	2478	1923
Ni	1.072	0.817	0.885	0.997	1.054
P	421	345	351	351	379
Pb	4.257	3.447	3.151	2.988	4.222
S	366	262	277	267	313
Se	0.300	0.810	0.631	0.833	0.286
Si	398	96	96	85	106
Zn	38.1	26	25.1	27.2	37.8

Table A4. Mean Stem Nutrient Analysis for Two-year Old Loblolly Pine in Response to Foliar Applied Oil (expressed in ppm).

Nutrient	0%	25%	50%	75%	100%
Al	138	152	163	171	168
B	20.07	16.63	14.08	15.45	11.29
Ca	1675	1723	1770	1686	1605
Cd	0.332	0.280	0.584	0.466	0.286
Co	0.146	0.126	0.152	0.168	0.218
Cr	0.863	1.397	1.130	2.970	1.617
Cu	3.237	2.772	2.604	3.191	2.291
Fe	62.21	58.03	108.22	235.93	98.83
K	987	973	1160	1457	1592
Mg	537	504	551	554	559
Mn	175	171	185	206	173
N	5723	4375	5040	5152	5464
Na	4102	2762	2877	3174	2343
Ni	1.022	0.897	1.291	2.460	1.322
P	495	431	474	556	581
Pb	5.142	5.205	5.177	5.208	7.194
S	375	298	339	374	407
Se	0.276	0.710	0.518	0.787	0.357
Si	396	142	134	127	135
Zn	46.14	34.00	31.54	38.22	46.74

Table A5. Mean Bole Nutrient Analysis for Two-year Old Loblolly Pine in Response to Soil Applied Oil (expressed in ppm).

Nutrient	0 L/ha	431 L/ha	862 L/ha	1724 L/ha
Al	141	63	114	99
B	10.44	5.30	6.59	6.93
Ca	1629	1517	1322	1316
Cd	0.201	0.151	0.172	0.180
Co	0.061	0.130	0.075	0.019
Cr	0.844	3.273	1.368	1.524
Cu	2.202	2.239	2.464	2.593
Fe	29.38	39.81	49.52	36.44
K	960	802	927	1227
Mg	506	401	439	471
Mn	141	114	96	81
N	5726	4330	4431	4472
Na	2332	1173	1109	939
Ni	0.852	2.956	0.914	1.123
P	401	231	291	375
Pb	2.445	2.424	2.677	5.568
S	335	239	281	290
Se	0.645	0.673	0.493	0.089
Si	95.43	49.27	75.76	55.57
Zn	33.71	27.65	20.76	27.51

Table A6. Mean Stem Nutrient Analysis for Two-year Old Loblolly Pine in Response to Soil Applied Oil (expressed in ppm).

Nutrient	0 L/ha	431 L/ha	862 L/ha	1724 L/ha
Al	109	93	130	127
B	11.466	8.569	9.142	9.351
Ca	1507	1408	1350	1394
Cd	0.198	0.121	0.150	0.141
Co	0.083	0.134	0.107	0.055
Cr	1.047	1.755	2.062	1.583
Cu	2.759	2.736	2.678	2.954
Fe	24.49	24.39	51.43	36.33
K	1290	1488	1789	2399
Mg	511	519	547	563
Mn	111	109	125	120
N	5128	5346	5842	5853
Na	2871	2345	1691	1301
Ni	1.132	1.767	2.697	1.547
P	492	402	485	604
Pb	2.764	4.475	9.033	10.487
S	354	341	374	400
Se	0.626	0.732	0.705	0.231
Si	96.42	85.36	95.59	76.97
Zn	40.40	42.84	30.41	35.10

Table A7. Mean Bole Nutrient Analysis for Two-year Old Loblolly Pine in Response to Foliar Applied Brine (expressed in ppm).

Nutrient	0%	25%	50%	75%	100%
Al	91.55	99.70	94.21	81.41	81.36
B	9.547	8.392	8.579	6.239	8.027
Ca	1363	1395	1366	1192	1360
Cd	0.157	0.182	0.193	0.029	0.160
Co	0.053	0.056	0.059	0.037	0.046
Cr	1.300	0.873	0.889	0.827	0.950
Cu	2.558	2.180	2.242	1.942	1.993
Fe	43.81	35.36	37.12	30.98	37.39
K	417	666	493	408	479
Mg	395	415	350	294	340
Mn	151	149	139	119	144
N	4486	3394	3686	2989	4054
Na	1633	1452	1254	1243	1846
Ni	0.766	0.757	0.657	0.478	0.680
P	227	258	229	188	241
Pb	1.994	2.262	1.617	1.678	1.266
S	211	217	192	153	213
Se	3.094	1.103	1.845	2.647	1.140
Si	76.77	62.02	71.63	55.03	56.67
Zn	25.94	22.37	18.13	17.33	20.65

Table A8. Mean Stem Nutrient Analysis for Two-year Old Loblolly Pine in Response to Foliar Applied Brine (expressed in ppm).

Nutrient	0%	25%	50%	75%	100%
Al	106	115	121	124	140
B	16.12	11.50	13.76	12.53	14.08
Ca	1399	1371	1453	1270	1658
Cd	0.169	0.200	0.161	0.051	0.175
Co	0.099	0.098	0.111	0.041	0.102
Cr	1.245	4.620	1.939	0.766	0.970
Cu	3.061	2.868	2.623	2.624	2.434
Fe	31.97	46.47	33.40	25.65	41.52
K	880	1014	1065	957	1194
Mg	431	429	443	369	485
Mn	195	160	185	162	213
N	0.571	0.463	0.522	0.411	0.518
Na	2704	2042	2277	2219	3371
Ni	0.867	3.476	1.139	0.547	0.880
P	405	354	384	318	418
Pb	3.416	3.057	3.215	3.783	3.318
S	306	246	281	236	334
Se	3.522	0.879	1.689	2.323	1.449
Si	113	90	106	82	109
Zn	38.02	28.98	26.07	26.03	37.65

Table A9. Mean Bole Nutrient Analysis for Two-year Old Loblolly Pine in Response to Soil Applied Brine (expressed in ppm).

Nutrient	0 mS/cm	2 mS/cm	4 mS/cm	8 mS/cm	16 mS/cm	32 mS/cm
Al	106	66	82	93	50	127
B	6.57	6.10	8.28	10.83	26.40	87.87
Ca	1443	1302	1397	1285	1053	1866
Cd	0.164	0.145	0.194	0.173	0.173	0.174
Co	0.076	0.068	0.128	0.120	0.149	0.343
Cr	0.695	0.740	0.778	0.993	0.887	0.683
Cu	2.012	2.242	2.231	1.886	2.315	2.817
Fe	32.84	24.01	34.73	34.39	22.25	27.71
K	496	546	597	573	675	1635
Mg	358	425	410	342	405	713
Mn	171	121	151	126	54	152
N	4040	4990	4436	3134	4906	4446
Na	1553	1537	1426	2508	5889	14851
Ni	0.458	0.635	0.605	0.432	0.531	0.762
P	170	267	318	150	272	561
Pb	3.047	1.693	2.231	1.221	5.853	2.672
S	217	244	224	217	299	449
Se	0.869	0.655	0.697	0.863	0.499	0.733
Si	71.60	50.61	67.80	64.71	43.28	81.87
Zn	16.43	20.29	14.83	14.71	20.33	28.79

Table A10. Mean Stem Nutrient Analysis for Two-year Old Loblolly Pine in Response to Soil Applied Brine (expressed in ppm).

Nutrient	0 mS/cm	2 mS/cm	4 mS/cm	8 mS/cm	16 mS/cm	32 mS/cm
Al	124	64	95	108	78	113
B	11.36	8.75	16.55	19.57	55.25	92.30
Ca	1536	1117	1397	1415	1412	1601
Cd	0.15	0.12	0.17	0.17	0.26	0.21
Co	0.12	0.09	0.18	0.11	0.20	0.36
Cr	0.73	0.57	0.56	1.23	0.72	0.69
Cu	2.72	2.52	3.20	2.53	4.11	3.49
Fe	26.27	14.40	18.52	23.54	19.41	22.00
K	1306	1056	1274	1525	2681	2237
Mg	449	444	491	447	766	752
Mn	215	132	189	187	166	126
N	0.51	0.60	0.55	0.54	0.88	0.66
Na	2585	2311	2612	5505	10226	17190
Ni	0.73	0.72	0.86	0.78	0.83	0.95
P	276	403	534	316	803	770
Pb	12.57	5.30	8.07	5.56	2.35	1.77
S	338	291	325	381	508	486
Se	0.77	0.63	0.54	0.74	0.65	0.57
Si	126.27	80.04	94.05	99.76	85.07	90.14
Zn	27.29	31.19	23.15	20.90	33.68	29.76

VITA

Dean Goodin was born in 1975 in Columbia, South Carolina. He grew up in Memphis, Tennessee, where he graduated from Christian Brothers High School. In 1993, he began his undergraduate studies at Louisiana State University in Baton Rouge, Louisiana, majoring in environmental management systems and minoring in agronomy and microbiology. He graduated with his bachelor of science degree in December 1997. In January 1998, he was offered a graduate research assistantship by the Louisiana State University Department of Agronomy to pursue his doctoral degree. He is a member of the Society of Wetland Scientists. He is currently a candidate for the degree of Doctor of Philosophy. He has accepted a position with BEM Systems, Inc. in Orlando, FL upon graduation.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Dean Anthony Goodin

Major Field: Agronomy

Title of Dissertation: Remediation of an Oil Contaminated Wetland and the Effects of Crude Oil and Brine on Two-year Old Loblolly Pine (Pinus taeda) Seedlings

Approved:

Wayne H. Hudnall
Major Professor and Chairman

[signature]
Dean of the Graduate School

EXAMINING COMMITTEE:

Allen E. [signature]

[signature]

Maud M. Walsh

Irving A. Mendelssohn

[signature]

Gary Breitenbeck

Date of Examination:

May 14, 2001